# Biological aspects of the decay of *Pinus sylvestris* leaf litter[1])

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## Abstract

The microfungi contributing to the breakdown of leaf litter of *Pinus sylvestris* were investigated by several techniques concurrently and a well-defined succession was worked out. Colonization of the interior and exterior of the needles is carried out by different groups of fungi. The fungi are largely responsible for the development of the subdivision of the organic horizon known as the $F_1$ layer. Subsequently the soil animals produce the condition recognized as the $F_2$ layer. Eventually, about 10 years after the needles have fallen from the tree their now comminuted remains enter the H or humus layer, where biological activity is greatly reduced.

## INTRODUCTION

The concept of succession is well established in plant ecology and many detailed studies of the seral communities have been made. These studies have nearly all been concerned with higher plants and although there are numerous brief references to fungal successions, few careful studies with any degree of completeness have been made.

Some of the most interesting fungal successions are those which arise on naturally occurring plant debris. Several studies of this type of succession have been made. CHESTERS (1950) investigated the ascomycetes inhabiting and contributing to the breakdown of many types of tree bark, while MANGENOT (1952) carried out more detailed work on the fungi concerned in the progressive decomposition of dead trunks of various deciduous trees. WEBSTER (1956, 1957) has done similar detailed cultural and observational work on the fungi inhabiting decaying culms of *Dactylis glomerata*, and more recently HUDSON and WEBSTER (1958) have followed a similar succession on *Agropyron repens*. PUGH (1958) investigated a fungal succession on leaf litter of

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*Carex paniculata*. Other workers have reported on the microflora of various types of plant debris at one stage in the decomposition process, and many thousands of isolated observations of fungi on decaying plant residues have been recorded, viz. Rabenhorst's Kryptogamenflora, but careful sequential observations are almost invariably lacking.

This study is concerned with the fungal succession on the leaf litter produced by *Pinus sylvestris* on a typical podzolised sandy soil. The litter decomposes to form typical mor humus, and although it has been recognized for many years that the fungi play a dominant role in such processes, our knowledge of the fungi concerned is very limited. HANDLEY (1954) in his excellent account of mull and mor has stressed how little is known of the microbiological processes involved. Many workers who have attempted fungal isolations from forest soils (e.g. ELLIS, 1940, THORNTON, 1956) did not consider the organic horizon at all. COBB (1932) treated the complex organic horizon as a single sampling unit, and made no attempt to devise suitable specialised isolation techniques. OHMASA, KAWADA and KAWADA (1957), while recognising subdivisions of the organic horizon, still relied on unsuitable methods of isolation.

In the area chosen for detailed study the accumulated plant debris and humus form a thick layer which can readily be separated into different stages in the succession which converts the freshly fallen needles into amorphous humus. The microflora is predominantly fungal; bacteria appear to play little part in the succession, perhaps because of the presence of antibiotics but more likely because of the predominantly acid reaction of the litter. The fungus flora of the leaf litter has been investigated with a view both to establishing the identity of the dominant darkly pigmented forms reputed to be present in mor-type organic horizons, and to following the course of the ecological succession in the leaf microsere.

## MATERIALS

The leaf litter chosen for this study was that of a pure stand of *Pinus sylvestris* in Delamere Forest, Cheshire, England. The bedrock underlying the podzol at Delamere is Keuper marl which is here overlain by a thick layer of glacial sand. Previous to the Enclosure Act of 1812 this area was common land, and it is thought that a cover of *Calluna vulgaris* existed. It seems probable that mor conditions with attendant podzolisation commenced under the *Calluna*, and were continued and intensified under the Scots pine after the land was afforested. It is estimated that the Delamere podzol has been in course of development for about two thousand years.

The stand studied was growing on a well-drained, gently sloping site at just over 80 metres above sea level, and consisted of approximately fifty-year-old trees produced as a result of natural regeneration in the years 1900–1908 and thinned five times since. The ground flora around the site from which samples were derived consisted almost entirely of *Pteridium aquilinum*. *Vaccinium myrtillus* was to be found nearby wherever the stand was less dense, and the only other species observed in this locality was *Deschampsia flexuosa*.

The organic horizon in Delamere Forest was the result of protracted accumulation of litter in various stages of decay, much of which, in the manner typical of mor, consisted of recognisable leaf remains. HESSELMAN (1926) suggested that mor-type organic horizons should be divided into three more or less distinct sub-horizons or layers. He recognised a surface layer of dead but apparently undecomposed leaf litter which he termed *Förna;* a second underlying layer of decomposing but still recognisable litter, which he named *Förmultningskiktet;* and a third layer of amorphous humus with few uncomminuted leaf fragments now remaining, which he called *Humusämneskiktet*. These are now commonly called the L or litter layer, F or fermentation layer, and H or humification layer. In cases of considerable accumulation the F layer may be further divided into $F_1$ and $F_2$ subhorizons (KUBIENA, 1953). At Delamere all these subdivisions were clearly recognisable (Fig. 1). The L layer was composed of recently fallen needles, light brown to buff in colour, and others, generally darker in colour, which had been on the ground for longer periods. All these had high tensile strength and relatively low moisture content, and lay in a loose uncompacted layer at the surface. $F_1$ needles were grey to black, with softened tissues, low tensile strength and relatively high moisture content, lying in a more closely packed stratum beneath the L layer. In the $F_2$ layer the needles had become greyish and were generally fragmentary. They were often flattened and lay in a tightly compressed mat below the $F_1$ layer. The H layer consisted largely of animal faeces and fungal remains, the needles having undergone complete physical reduction. Mycorrhiza of pine were sometimes found in this layer, which lay immediately above the humus stained $A_1$ horizon of the mineral soil. No absolutely sharp dividing line could be drawn between adjacent subhorizons, as transitional stages were naturally in evidence, but a very high proportion of all needles could invariably be assigned to one of the main categories just described.

An examination of the organic horizon at Delamere showed that its depth and composition varied considerably according to the contour, plant cover and time of year. The most variable component was the

$F_1$ layer which, while completely absent from some localities, attained a thickness of up to 15 cm. around the bases of certain trees, and was only slightly less thick in some dense patches of *Pteridium aquilinum*, in this case largely owing to the presence of many bracken remains which appeared very resistant to decay. As the object of this study was to investigate the decomposition of litter derived from a single plant species, sampling sites were chosen carefully to exclude locations with *Pteridium*. It was important to obtain samples from sites on which all layers of the organic horizon were adequately represented, and to this end locations were always selected quite subjectively.

The advantage of studying deep pine litter lay not only in the easily recognisable progressive stages of decay, but also in the existence of a habitat micro-unit, the pine needle, which could be transported to the laboratory, and there subjected to various experimental procedures, without loss of integrity. This allowed a much clearer picture to be built up of fungal distribution within the substrate, and permitted the derivation of meaningful quantitative and qualitative data, which could be interpreted in terms of a basic natural unit. This had not been possible with the majority of habitats previously investigated from a microbiological standpoint by other workers.

## METHODS

Although it would be ideal if a single non-selective technique could be devised which would permit the isolation of the entire fungal flora of a given habitat, this is not likely to be achieved and, in recent years most workers, realising this, have used several specialised techniques concurrently in the study of individual problems. At present the most frequently adopted compromise is the parallel application of direct and indirect techniques.

The techniques devised or applied during this study had the advantage that the fungi isolated could either be observed during isolation, or could often be presumed to be present in the substrate in an active mycelial condition. The direct techniques employed also ensured the recognition of certain forms which, though shown to be active in the habitat studied, did not lend themselves to artificial culturing.

In the early part of this work a series of dilution plates and soil plates prepared from the H layer of the organic horizon yielded a small number of fast-growing, heavily sporing hyaline forms, chiefly *Penicillium* species and *Trichoderma viride*. Direct observation of suspensions prepared from the H layer revealed large numbers of darkly pigmented hyphal fragments. It was clear that although these were predominant in the H layer, they were not represented in the colonies appearing on the plates. In an attempt to resolve this inconsistency a hydraulic micromanipulator was employed to pick out several hundred of the dematiaceous fragments, and these were individually plated on various nutritive media and periodically examined, but invariably failed to produce colonies. Further direct observation of those layers of the organic horizon overlying the H layer gave the answer to this problem. It soon became clear that a large proportion of the dark fragments had originally developed as part, either of the surface reticula of hyphae to be seen on most needles of the $F_1$ layer, or of their accompanying conidiophores. In the $F_2$ layer, the hyphal network and conidiophores had apparently been largely removed by some agency. Examination of the droppings of the soil fauna suggested that these minute animals were largely responsible for this phenomenon. It was now obvious that in order to provide as complete a picture as possible of fungal activities in the L, $F_1$ and $F_2$ layers, these would all have to be investigated concurrently.

Differentiation between actively growing mycelia on or in the substrate, and dormant spores borne passively on the surface was achieved in this work by a technique adapted from one developed by HARLEY and WAID (1955) for the study of the microflora of beech roots. Discrete segments of root were shaken in many changes of sterile water to remove surface-borne spores. The efficacy of the washing was assayed by pouring dilution plates with samples of the washing water taken after increasing numbers of washings. In the present work, root segments were replaced by entire single needles of *Pinus sylvestris*. Preliminary experiments showed that even twenty-five washings in plain water were insufficient to ensure adequate removal of detachable propagules. It was therefore decided to use a dilute solution of detergent in the hope of increasing the effectiveness of the washings to a level where a practicable number of washings would produce a relatively propagule-free surface. Accordingly the first ten washings were carried out in 1 % 'Teepol' solution, and a further ten in distilled water. The detergent proved strikingly successful, and even five washings left far fewer propagules than twenty-five washings in plain water. The use of detergent had no observable effect on the development of fungi from needles plated on nutrient media subsequent to washing.

In the experimental procedure finally adopted for serial experiments, forty needles of each sampling type were treated. They were washed in separate one-ounce screw-top phials, in serial changes of 20 ml. of sterile liquid, each washing having a duration of two minutes. During the washings, the phials were shaken at a frequency of 300 oscillations per minute, through a distance of 4 cm. Five 1-ml samples were taken from the phials containing A, L and $F_1$ needles at the conclusion of the fifth, tenth and twentieth washings, a total of 45 dilution plates being prepared using 2% malt agar at pH 5.0. Table 1 shows the relative efficiency of the method as applied to needles of the different layers. The technique was clearly very effective for the treatment of living needles and those of the L layer. However, in the $F_1$ layer, and presumably also in the $F_2$ the physical condition of the needles appeared to preclude complete removal of spores. In order to obtain a fairly complete picture of fungal activities in these layers it was necessary to correlate data obtained from a combination of techniques.

Table 1

Washing technique—comparison of efficiency with different needle types.

| | Propagules removed per needle | | |
|---|---|---|---|
| Needle type | washing 5 | washing 10 | washing 20 |
| A | 0 | 0 | 0 |
| L | 6 | 1 | 0.25 |
| $F_1$ | 65.9 | 31.5 | 15.6 |

The second important differentiation which had to be made in analyzing the pine needle microflora was that between active mycelia on the surface of the needle and those growing within its substance. This was attempted by employing a surface sterilization technique. Davies (1935) and others have shown that the toxicity of various chemicals to different fungal genera varies so much as to constitute a means for the differential isolation or control of certain forms. In the present work it was considered that the most suitable agent would be that which was least specific in its action. A pilot experiment was run, using several common sterilizing agents, and as a result, a 0.1% aqueous solution of mercuric chloride was adopted for all subsequent work, and the optimal time of immersion in this sterilizing fluid was found to be two minutes.

All media are known to be more or less selective in certain respects, depending on their reaction and differing content of the various groups of nutrients. Here it was hoped to employ this selectivity in the isolation of fungi possessing dark pigment. In comparative experiments with several common laboratory media and a specially compounded pine needle agar, the most suitable medium for cultural work with the litter proved to be 2% malt extract agar at pH 4.5. This medium consistently showed a higher dominance of dematiaceous forms, and a lower incidence of bacteria, than any of the other media employed.

In addition to plating the variously treated needles on a nutritive medium, it was considered desirable to hold corresponding samples in sterile conditions with a high humidity, in the absence of nutrients outside the needles. This, it was hoped, would induce the fruiting of various fungi present on or in the needles, but unable to compete on nutritive media with other fast-growing forms. For this purpose, damp chambers were set up, consisting of petri dishes around whose inner periphery asbestos cord, saturated with water, was wound prior to sterilization. This ensured the maintenance of a high humidity within the dish for several months, and enabled a number of fungi not revealed by other cultural techniques to be observed, quite often in the fruiting condition.

In order to supplement the information gained from the cultural studies, direct microscopical examinations were made of large numbers of individual needles from each layer. These observations, originally designed to be of a supplementary nature only, were soon recognised to be of great significance, and have been fully documented.

The foregoing procedures were used in an extended investigation of the fungus flora of the various needle types throughout the year. It was thus hoped to discover, at least in part, the nature of the sequence of fungi participating in the colonization and breakdown of the needles, and to ascertain the influence of environmental conditions on these fungi.

## RESULTS – CULTURAL STUDIES

Observations and isolations were made at the end of January 1957 and on eleven subsequent occasions. Needle samples of the various types described above were collected in sterile containers and brought back to the laboratory. Batches of 40 single needles of each of the four main types, A, L, $F_1$ and $F_2$ were selected and placed in 1-ounce screw top phials. They were then subjected to the washing technique described above. After washing, each batch of 40 needles was treated as follows; 20 were plated on 2 % malt extract agar, four to a plate, and 20 were similarly distributed between five damp chambers. A further 40 needles of each type were surface sterilized, and these were distributed in exactly the same manner as the washed needles. Thus 320 needles were treated in each experiment.

The results of the cultural studies are presented in Figures 2 to 9. The graphs show the fluctuations in frequency of isolation of the seven most important genera in the several layers and at different times of year. More than 70 species were isolated during the work, but, apart from those recorded in the graphs, none ever occurred with sufficient frequency to be accounted important in the habitat.

Striking variations in the percentage frequency of occurrence of the seven main genera were revealed by the experiments. These fluctuations existed both between the different layers of the organic horizon in any given month, and in each layer from season to season. The results obtained by the surface sterilizing technique also differed greatly from those given by the washing technique. These differences were largely quantitative rather than qualitative, certain fungi showing increased frequency of occurrence after surface sterilization, while other species displayed correspondingly reduced incidence. These results allow a composite picture to be built up of some important aspects of the fungal succession. That this succession had other major fungal components was demonstrated by other techniques which are discussed in later sections of this paper.

The results for each type of needle are discussed below and are represented graphically in Figures 2 to 9.

Living A needles. Figures 2 and 3 show that *Coniosporium* was the form most frequently isolated. It is also apparent that surface sterilization of needles prior to culturing considerably reduced the number of isolations of this fungus. This may indicate that the species was a surface-dweller. In those cases where it survived immersion in the sterilizing fluid, it is possible that the substomatal cavities had been colonized, enabling the fungus to persist. The fungus, though of frequent occurrence on living needles, did not colonize the surface very extensively, as only one or two rather restricted colonies usually appeared on a single needle.

*Pullularia pullulans* and *Fusicoccum bacillare* were the only other fungi to attain significant frequencies of occurrence on living needles. Where they did occur, their mycelium grew out from considerable portions of the length of the needles. The graphs show that these fungi were, respectively, infrequent and completely absent in winter and spring, being isolated mainly between May and September. These were the months preceding and including the main seasonal needle fall, and it seems probable that the needles from which *Pullularia* and *Fusicoccum* were isolated were senescent. After these needles had fallen in August or September, the incidence of these two fungi on living needles would automatically be reduced. It is noticeable that the incidence of *Pullularia* decreased considerably after surface sterilization, suggesting that this fungus, like *Coniosporium*, was mainly a surface colonizer.

Layer L needles. (Figures 4 and 5). The impression that *Pullularia*, one of the commonest fungi on washed L needles in culture, was largely a surface colonizer, was confirmed by the way in which it was partially suppressed by surface sterilization. *Pullularia* was found on a high proportion of L layer needles all round the year in a potentially active condition, and was considered to be one of the primary saprophytic colonizers of the needles after the death of the tissues. This fungus is of very widespread occurrence. SMIT and WIERINGA (1953) isolated it from recently dead leaves of various deciduous trees, and MELIN and NANNFELDT (1934) obtained numerous cultures of this species from air, water and woodpulp. It has been suggested by HADDOW (1941) that *Pullularia* may be parasitic when introduced into wounds caused by insects.

When the annual needle fall occured just before the end of August, 1957, it was decided that a distinction should be made within the L layer between freshly fallen 'new' needles, and those which had been lying on the surface for several months prior to being covered by the new deposit of litter, 'old' needles (Fig. 10). These subdivisions of the L layer, which were naturally much more clearly marked at this time of year than at any other, showed sharp quantitative and qualitative differences in their microfloras. Freshly fallen needles gave a high incidence of *Pullularia*, while 'old' needles showed a great reduction in the occurrence of this fungus within a few weeks of being covered by the new fall. With the change in conditions in the habitat, the next phase of the succession soon developed. This was marked by the entry of other surface forms with, perhaps, less tolerance of desiccation, but able in other ways to compete successfully with *Pullularia*, and even possibly to produce conditions inimical to that fungus. The second form seen to be of particular importance in the L layer was *Fusicoccum bacillare*. This species occured on about 40 % of all L needles washed and plated on nutrient agar. However, when surface sterilized needles were considered, the relative incidence of *Fusicoccum* was seen to have increased. This may have been a sign of reduced competition from *Pullularia* on the culture medium, but in view of the generally non-selective action of mercuric chloride, it was also taken as an indication that *Fusicoccum* inhabited the interior of the needles, and not merely their superficial layers. As in the case of *Pullularia*, a high percentage occurrence of *Fusicoccum* was found on newly fallen needles, and a rapidly falling incidence on 'old' needles.

Samples of needles which, though dead, had not yet fallen from the tree, were collected and subjected to the standard experimental procedures. These revealed very high frequencies of occurrence for *Pullu-*

*laria* and *Fusicoccum*. It thus appeared that these two fungi were able to colonize recently dead needles while these were still on the tree. No other fungi were recorded from these samples, and it must be concluded that any other fungi found on needles of the L layer were secondary colonizers, becoming competitive only after the needles had fallen.

A case in point was *Desmazierella acicola*, the third species isolated with fairly high frequency from the L layer. This fungus, unlike *Pullularia* and *Fusicoccum*, did not attain maximum incidence in needles of the surface layer, a fact of some successional significance which is further discussed below. Surface sterilization produced an appreciable increase in the occurrence of *Desmazierella* in several months; suggesting once more that this species inhabited the interior of the needles. This increase was not as marked as that for *Fusicoccum*, suggesting that *Desmazierella* was not, unlike *Fusicoccum*, adversely affected by competition from *Pullularia* in culture. Needles which had fallen in the months immediately preceding sampling showed a very low incidence of *Desmazierella*, while 'old' needles gave a much higher frequency of occurrence. This was a trend exactly opposite to that observed for *Pullularia* and *Fusicoccum*, and seemed to provide evidence for the establishment of another stage in the succession.

Layer $F_1$ needles. (Figs. 6 & 7). This evidence was amply supported by the results of cultural studies of the $F_1$ layer, which revealed a very different situation from that existing in the L layer. The washing technique gave an apparent reduction in incidence of *Desmazierella* as compared with the L layer, while occurrence of *Trichoderma viride* rose sharply. Surface sterilization exposed the inadequacy of the washing technique in this case, and, by producing a striking rise in the incidence of *Desmazierella* with an equally marked reduction in that of *Trichoderma*, showed something of the real importance of the former in the interior of the $F_1$ needles. It was clear that *Desmazierella*, while relatively unaffected by competition from *Pullularia* and *Fusicoccum* in culture, was somewhat inhibited by the almost universal presence of *Trichoderma* on washed needles of the lower layers. *Trichoderma* existed either as numerous spores or as mycelium on the surface of these needles, and the porous nature of this surface unfortunately seemed to prevent the removal of all spores by the washing technique. A similar effect was observed with the *Penicillium* species existing in this layer, and with *Mucor hiemalis*, which, though only sporadically isolated from needles of the L layer, was quite common on washed $F_1$ and $F_2$ layer needles. This species was completely eliminated by surface sterilization. *Pullularia* and *Fusicoccum* were isolated from only a small proportion of $F_1$ needles, and were obviously now only of minor importance. Another stage of the fungal succession had become firmly established, with *Desmazierella* and *Trichoderma* as the dominant forms.

Layer $F_2$ needles. (Figs. 8 & 9). In considering the isolations obtained from needles of this layer, four fungi stand out. These are *Desmazierella acicola*, *Mucor hiemalis*, *Trichoderma viride* and *Penicillium* species. Here again the washing technique was presumably ineffective, as washed needles from this layer showed *Trichoderma* to be completely ubiquitous, and to suppress growth of *Desmazierella* almost completely, only *Penicillium* and *Mucor* being able to grow alongside it in culture. The surface sterilization technique did produce a considerable shift in the balance toward *Desmazierella* and away from *Trichoderma* and *Penicillium*, but though the time of immersion in the sterilizing fluid was reduced to one minute, the porous nature of the decomposing tissues allowed too much penetration of the fluid, and produced an overall reduction in the number of isolations.

The use of damp chambers proved interesting, but rather disappointing, as conditions apparently failed to simulate those of the natural habitat, and many forms seen by direct observation were not often selected for by this method. Living needles, and those of layer L, commonly gave rise to dark mycelia, which were shown to be of *Pullularia*. In the $F_1$ layer, although dense growths of *Sympodiella acicola*, *Helicoma monospora*, and *Desmazierella acicola* were sometimes seen, more usually no regular pattern of occurrence could be discerned. A number of interesting fungi were, however, isolated, many of which are known to attack cellulose, and most of which were dematiaceous. They included species of *Oidiodendron*, *Stysanus* and *Stachybotrys*, which have frequently been observed by Tribe (1957) as primary colonisers of cellulose films buried in soil. Needles of the $F_2$ layer when surface sterilized usually showed no trace of fungal growth in damp chambers. Washed needles, however, showed a high incidence of a sterile dark mycelium.

## RESULTS - DIRECT OBSERVATION

Direct observation has assumed, in the present investigation, an importance equal to that of the cultural studies detailed above. Certain interesting fungi not revealed by cultural techniques have been shown to be of considerable importance in the fungal succession on the decaying needles, and it was found that useful quantitative data on seasonal fluctuations in these fungi could be obtained by sequential observations.

Freshly collected needles of the categories previously described were examined in the direct illumination of a spotlamp under a high-power binocular dissecting microscope. Normally one hundred needles of each type were examined, and the incidence of each fungus expressed as a percentage. Some of the species concerned produced conidiophores on the needle surface or growing out from the sub-stomatal cavities. In these cases the numbers of such conidiophores on each needle were estimated to the nearest power of ten. A review of direct observations for April 1957 is given below, and is followed by an account of the seasonal fluctuations in frequency of occurrence and in numbers of conidiophores per needle of the different species.

Living needles. Traces of dark hyphae were seen on 30 % of living needles removed directly from the tree and examined in April. These mycelia were probably those of *Pullularia* or *Coniosporium*. Small brown discoloured areas were observed on 23 % of living needles, possibly caused by infection of *Lophodermium pinastri*.

Layer L needles. In this layer, the form most conspicuous on direct observation was *Lophodermium pinastri*. This is a parasitic fungus which does not easily lend itself to artificial culturing (JONES, 1935). It infects the living needles of *Pinus sylvestris* in spring by means of ascospores, and its depredations sometimes cause a premature needle cast. The ascocarps, (hysterothecia), are black, elliptical structures, opening by a longitudinal slit and developing generally after the needles have fallen (Fig. 11). Finally, more ascospores are discharged in the spring. A peculiarity of this fungus is its formation of 'diaphragms', which show externally as a black ring around the needle. These diaphragms are purported to mark the limit of growth of the fungus, but their real significance is uncertain. Direct observation showed traces of *Lophodermium* infection in 41 % of L layer needles, 24 % exhibiting only the characteristic diaphragms, the other 17 % bearing hysterothecia. This fungus is believed to be an extremely important primary colonizer of the interior of the pine needles, and one which has a strong influence on the subsequent course of the decomposition process.

The species most obvious after *Lophodermium* were two small black carbonaceous pycnidial fungi, the more frequently observed of which was *Fusicoccum bacillare*, the other being *Sclerophoma pithyophila*, thought to be a stage in the life history of *Pullularia pullulans* (BATKO, MURRAY and PEACE, 1958). These pycnidia occurred on 35 % of L layer needles, of which 4 % bore in excess of 100 pycnidia each, a good indication of the density of colonization which was sometimes found. 12 % of the needles showed a localized blackening in and around the stomata, and sometimes tiny stromatic masses of fungal tissue protruded from the stomates. These tiny black spots, arranged in linear series, gave a very characteristic appearance to the needles. They were finally recognized as the sites from which the conidiophores of *Verticicladium trifidum*, the imperfect state of *Desmazierella acicola*, would eventually develop. In fact a further 3% of the needles bore developing conidiophores of this fungus, bringing the total observed incidence to 15 %. The ascigerous stage of *Desmazierella acicola* was never observed during this study.

Less conspicuous and characteristic than the above were the dark hyphal masses found on 44 % of the needles. These mycelia were thought to be those of *Pullularia pullulans* in particular. The only other fungal phenomenon observed with significant frequency in the L layer was the development of traces of superficial dematiaceous hyphal networks on 67 % of the needles (Fig. 12). These reticula were those of two new species of hyphomycete discussed below, but to distinguish between them at this stage was impossible, as no conidiophores were present.

Layer $F_1$ needles. The main development of hyphomycetes occurred on needles of this layer. Here conidiophores of several fungi not observed on nutritive media were seen. The three most important forms fruiting in this layer were *Helicoma monospora* (Fig. 13), *Sympodiella acicola* (Fig. 15) and *Desmazierella acicola* (Fig. 16). *Helicoma monospora* and *Sympodiella acicola* were previously unknown, and have been designated as a new species and a new genus respectively (KENDRICK, 1958a, 1958b).

Detailed observations were made of the progress of these fungi in the $F_1$ layer. A complete analysis of the observations made in April 1957 is given in Table 2, as this was one of the months of maximum fungal activity. The needles of the $F_1$ layer could be separated visually into three categories. These were labelled dark grey, grey-black and black, and represented successive stages in the colonization of the needles by various fungi. Needles were classed, as mentioned above, as to whether they bore less than ten, between ten and 100, between 100 and 1,000, or over 1,000 conidiophores of each species. The massive complex conidiophores of *Desmazierella* never exceeded 1,000 in number on a single needle, but the minute simple conidiophores of *Helicoma* and *Sympodiella* on occasion exceeded 10,000 to one needle, and numbers in excess of 1,000 were common. Table 2 shows the percentage occurrence of the different classes of conidiophore density on each of the three needle types of the $F_1$ layer.

It is quite clear that in each case there were two pronounced trends. The first and more marked of the two was toward the development of

Table 2

| Fungus | Conidiophores per needle | Dark grey % | Needle type Grey-black % | Black % | Total $F_1$ % |
|---|---|---|---|---|---|
| *Helicoma monospora* | $>10^3$ | 6 | 16 | 60 | 27 |
| | $>10^2$ | 28 | 64 | 35 | 42 |
| | $>10$ | 55 | 5 | 3 | 21 |
| | Total occurrence | 89 | 85 | 98 | 90 |
| *Sympodiella acicola* (developing) | $>10^3$ | 2 | 36 | 39 | 26 |
| | $>10^2$ | 23 | 45 | 55 | 41 |
| | $>10$ | 26 | 11 | 0 | 12 |
| (mature) | $>10^3$ | 0 | 3 | 3 | 2 |
| | $>10^2$ | 0 | 5 | 35 | 13 |
| | $>10$ | 10 | 13 | 3 | 9 |
| (dead) | $>10^3$ | 3 | 8 | 33 | 5 |
| | $>10^2$ | 3 | 37 | 55 | 32 |
| | $>10$ | 29 | 16 | 3 | 16 |
| | Total occurrence | 51 | 92 | 94 | 79 |
| *Desmazierella acicola* | $>10^2$ | 0 | 3 | 24 | 9 |
| | $>10$ | 10 | 29 | 58 | 32 |
| | $\pm 10$ | 45 | 55 | 18 | 30 |
| | Total occurrence | 55 | 87 | 100 | 81 |

larger and larger numbers of conidiophores of all three fungi as the needles gradually blackened and moved down in the $F_1$ layer. In fact in many cases the conidiophores of all three fungi, and the hyphal networks of *Helicoma* and *Sympodiella*, (Figs. 13, 14) played a substantial part in the darkening of the needle surface. The second shift was toward a greater percentage frequency of occurrence of each fungus, particularly well-marked in the cases of *Sympodiella* and *Desmazierella*, and by the time the needles were black, most were colonized to some extent by all three fungi. On any particular area of needle surface either *Helicoma* would be dominant, and *Sympodiella* almost absent, or vice versa. These two fungi appeared to be largely surface colonizers and were therefore to same extent in competition with each other, but no zones of mutual or unilateral inhibition between them have been observed.

*Desmazierella* conidiophores, arising from the substomatal cavities, were unaffected by the distribution of *Helicoma* or *Sympodiella*, but were strongly influenced by the presence of *Lophodermium* diaphragms.

These structures frequently appeared to mark the limit of colonization of the needle by *Desmazierella*, and numerous conidiophores of that fungus were often produced at the site of a diaphragm (Fig. 17). These diaphragms also frequently marked the limit of blackening of a needle, indicating that adjacent parts of the needle were at quite different stages of breakdown. Although blackening has been attributed in part to the reticula and conidiophores of the hyphomycetes, it was often almost entirely due to the production of pigment by internal colonizers, in particular *Desmazierella*.

Long straight hyphae of a sterile dark fungus were found on 26 %, and strands of basidiomycete mycelium on 16 %, of $F_1$ needles, but both were observed with greater frequency in the next lower layer, the $F_2$. Empty pycnidia of *Fusicoccum* were seen in 26 % of $F_1$ needles, and this fungus now appeared to have been completely superseded.

Layer $F_2$ needles. Observation of $F_2$ needle fragments in April showed that 92 % of these bore, usually in their concave surfaces, accretions of dark amorphous material. A squash preparation of this showed it to be a mélange of various types of organic debris. Most obvious were brown septate fungal hyphae, fragments of varying length, thickness, ornamentation and degree of pigmentation being present. Recognizable fungal remains included large numbers of fragmentary *Sympodiella* conidiophores, and conidia of *Helicoma*. Present in smaller numbers were the massive conidiophore stems of *Desmazierella*. Septate hyaline hyphae were also present, and included a high proportion of clamp-bearing basidiomycete mycelia. These accumulations comprised the faecal masses of the ectophagous meiofauna-enchytraeid worms, dipterous larvae, Acarina, and Collembola. With the faeces were various arthropod exoskeletal fragments and numerous minute nematodes. The stems of *Sympodiella* conidiophores remained on 39 %, and diaphragms or empty ascocarps of *Lophodermium* were seen on 40 %, of $F_2$ needles. Signs of obvious fungal activity in this layer were the numerous hyaline, threadlike, septate hyphae, possibly of *Trichoderma* or *Penicillium*, which were found on 73 % of the needles. Basidiomycete mycelium, frequently in the form of multihyphal strands was observed on 61 % of $F_2$ needles, and an incidence of 87 % was recorded for a sterile dark mycelium which grew across the surface of needles, and between adjacent fragments.

The H layer. This layer consisted largely of masses of meiofaunal faeces, and dark fungal hyphae and conidiophores (Fig. 18) resembling the accumulations found on the concave surfaces of $F_2$ needles. The H layer had a pH of 3.1, and apparently contained little suitable substrate for fungi. It thus seems that, without the disturbing influence of a soil macrofauna, particularly earthworms, microbiological activity had fallen, in this layer, to a very low level.

Having thoroughly examined the position in April, a month of intense fungal activity, a review of further direct observations of large needle samples which were carried out in other months is given below.

Living needles. Examination of living needles in other months showed no significant differences from the observations of April.

Layer L needles. The almost complete absence of *Lophodermium* diaphragms from needles freshly fallen at the end of August was noted with interest. These apparently did not form until the needles had been on the ground for some time. A similar phenomenon was observed with regard to the fruiting bodies of *Fusicoccum*. Ascocarps of *Lophodermium* actively produced ascospores only from March to June, and afterwards presented a rather flattened, dried-up appearance. The other fungus conspicuous in the L layer was *Desmazierella*, whose conidiophores matured mainly in the months June to September. This observation agreed with that of Hughes (1951). Figures for this layer are given in Table 3.

Layer $F_1$ needles. In the $F_1$ layer, sporulating conidiophores of *Desmazierella* were found between May and November, with maximum

Table 3

Direct observation—Incidence of importent forms in the L layer.

| Month | Lophodermium pinastri | | Fusicoccum bacillare | Desmazierella acicola | |
|---|---|---|---|---|---|
| | present % | sporing % | | present % | sporing % |
| 1957 | | | | | |
| March | 35 | 22 | 27 | 5 | — |
| April | 41 | 17 | 35 | 3 | — |
| May | 42 | 20 | 30 | 3 | — |
| June | 39 | 6 | 37 | 11 | 9 |
| August | o 43 | o — | o 34 | o 17 | o 5 |
| | n 3 | n — | n 6 | n — | n — |
| September | o 41 | o — | o 31 | o 12 | o 8 |
| | n 2 | n — | n 15 | n — | n 2 |
| November | o 38 | o — | o 39 | o 9 | o — |
| | n 15 | n — | n 28 | n 4 | n — |
| 1958 | o 39 | o — | o 32 | o 13 | o — |
| March | n 10 | n 4 | n 34 | n 12 | n — |

o denotes 'old' needles of the L layer

n denotes 'new' needles, added to the L layer by the seasonal needle fall incidence between June and August. With regard to *Helicoma* and *Sympodiella*, the figures presented in Table 4 show a maximum production of new conidiophores in the spring months, and there is reason to believe that the reduction in observed conidial numbers in autumn was due to the grazing activities of the mycophagous meiofauna, which according to OWEN EVANS (1955) and NIELSEN (1955) attain high numerical population strength at that time.

Table 4

Direct observation—Incidence of different conidiophore densities of three fungi on needles of the $F_1$ layer. March 1957—March 1958

| Month | Conidiophores per needle—% frequency of occurrence | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Helicoma monospora | | | | Sympodielle acicola | | | | Desmazierella acicola | | | | |
| | $>10^3$ | $>10^2$ | $>10$ | Total | $>10^3$ | $>10^2$ | $>10$ | Total | $>10^2$ | $>10$ | $\pm 10$ | Mature | Total |
| 1957 | | | | | | | | | | | | | |
| March | 29 | 48 | 15 | 92 | 28 | 45 | 16 | 89 | 10 | 49 | 30 | — | 89 |
| April | 27 | 42 | 21 | 90 | 26 | 41 | 12 | 79 | 9 | 32 | 40 | — | 81 |
| May | 42 | 36 | 8 | 86 | 35 | 26 | 21 | 82 | 6 | 26 | 39 | 5 | 71 |
| June | 37 | 43 | 14 | 94 | 37 | 23 | 14 | 74 | 20 | 46 | 23 | 37 | 89 |
| August | 23 | 50 | 10 | 83 | 30 | 43 | 4 | 77 | 17 | 52 | 14 | 29 | 81 |
| September | 15 | 27 | 33 | 75 | 30 | 44 | 6 | 80 | 24 | 36 | 15 | 21 | 75 |
| November | 12 | 22 | 34 | 68 | 19 | 37 | 16 | 72 | 11 | 43 | 20 | 9 | 74 |
| 1958 | | | | | | | | | | | | | |
| March | 22 | 41 | 9 | 72 | 15 | 39 | 15 | 69 | 14 | 43 | 27 | — | 84 |

Layer $F_2$ needles and the H layer showed no significant seasonal variations.

Direct observation has provided invaluable supplementary information to that obtained from cultural studies. Several of the fungi commonly observed were not isolated by the techniques employed for the periodic isolations. *Lophodermium* was perhaps the most significant example of this, but *Sympodiella* and *Helicoma* were only isolated by the direct plating of conidia on suitable media. Without the information gained by direct observation an incomplete and misleading picture of the fungal succession would have been obtained.

## NEEDLE SECTIONING

Although direct observation and cultural studies provided much information concerning the fungal succession which could only have been obtained by such an integrated approach, it was thought that useful confirmatory, and even additional, data could be derived from orthodox embedding and sectioning techniques. These, though too laborious to apply to the large samples necessarily involved in the other methods, could with advantage be used in special cases. Consequently, individual needles from each of the layers of the organic horizon were carefully selected by microscopical examination. These represented simple and mixed infections of the more important fungi concerned in the breakdown process, at various stages in their life histories. The chosen needles were fixed in Navashin's fixative (Formalin acetic alcohol), then embedded in 54°C paraffin wax. Sections were cut at 10 μ, and were examined without prior staining. This enabled a clearer picture of the processes leading to the blackening of needles to be obtained.

Layer L needles. Examinations of needle sections from this layer emphasized the importance of *Fusicoccum* as an internal colonizer in this layer. Mature pycnidia full of typical spores were present in needles which appeared to have been on the ground for a short time. These pycnidia developed completely immersed in the mesophyll, and their walls were composed of several layers of black fungal cells. At maturity they formed a narrow neck of similar tissue which pierced the host epidermis to allow spore discharge (Fig. 19). Occasionally two or three chambers of a 'spuriously plurilocellate' (GROVE, 1935) pycnidium were traversed by a single section, and sometimes .... separate pycnidia would appear on one section (Fig. 19). Associated with these pycnidia were brown septate hyphae, both inter- and intra-cellular, which riddled the mesophyll, frequently concentrating around the lysigenous canals, but did not appear to enter the stele at this stage. In 'old' needles affected by the same fungus, however, the phloem showed some signs of colonization. Sections of 'old' L layer needles exhibiting diaphragms of *Lophodermium* revealed that these diafphragms consisted of a band of host cells, extending across the entire width of the needle, which had been completely infiltrated by dark, thick-walled fungal elements. These grew both inter- and intra-cellularly, completely filling the lumen of the host cells and diffusing dense pigment into their walls (Fig. 21). It is of interest to note, however, that the thick cuticle overlying the epidermal cells remained colourless, even when the epidermis was so heavily pigmented as to be completely opaque in sections of 10 μ thickness. It seems very probable that, after the death of the *Lophodermium*, these diaphragms could constitute an impenetrable barrier to the growth along the needle of subsequent colonisers, a situation further discussed below. Sections through L layer needles bearing hysterothecia of *Lophodermium* (Fig. 20) showed that these were initiated between the epidermis and the hypodermis of the needles, but that later the epidermal cells became detached from the cuticle, and this became underlain by several layers of black stromatic fungal cells which formed the external covering of the fruit bodies. Internally the ascocarps were delimited by similar elements, which surrounded and invaded the hypodermis of the needle. The site of the future longitudinal dehiscence slit was marked by a reduction in pigmentation of the roofing elements. Breakdown of the needles was here restricted to the sites of the hysterothecia and the mesophyll, where many hyaline hyphae were seen.

In some needles, colonized usually by either *Lophodermium* or *Fusicoccum*, minute accumulations of dark fungal tissue could be seen filling substomata cavities (Fig. 22). These were thought to be the primordia of the stromata from which the conidiophores of *Desmazierella* would later arise. Colonization of the exterior of needles in the L layer appeared rather restricted. Traces of dark mycelia, presumably those of *Pullularia*, were seen in the shallow longitudinal grooves in which the stomata are situated, and in the angles of the leaf, particularly on the concave side of the needles.

Layer $F_1$ needles. Needles from the $F_1$ layer also showed differences in the degrees to which they had been attacked, and in the fungi which had colonized them. Pycnidia of *Fusicoccum* and ascocarps of *Lophodermium* encountered in $F_1$ needles were empty, and these fungi had apparently been supplanted by others. The darkening in colour which is so characteristic of needles in this layer was seen to be due to internal and external factors, either singly or in combination. The species mainly responsible for internal darkening was *Desmazierella acicola*, whose conidiophores had already been recorded from a high proportion of needles in this layer.

In some sections, a narrow black band of fungal tissue and stained host cells delimited areas colonized by *Desmazierella* (Fig. 23). On one side of this zone, all the host tissues were riddled with hyaline hyphae, and showed considerable breakdown. In addition, dark hyphae grew in and around the cells of the hypodermis and epidermis, staining their walls dark brown, and forming small stromata which emerged from the stomates. From these stromata, *Desmazierella* conidiophores frequently developed in this layer, sometimes occurring in small clusters. On the other side of the black zone the host tissues were relatively undecomposed, no darkening of the epidermal cell walls was present, and little trace of fungal colonization could be seen.

Sometimes the barrier created by a *Lophodermium* diaphragm marked the limit of colonization by *Desmazierella*, and thus a sudden transition from black to grey might be seen on direct observation of the needle (Fig. 17). Often, however, *Desmazierella* infections were present on both sides of a diaphragm, and caused complete blackening. Even in the absence of *Desmazierella*, the external hyphal networks of *Helicoma* and *Sympodiella* darkened the needle quite effectively (Figs. 14, 24).

In the $F_1$ layer a breakdown of the phloem tissues was observed, and needles which had been in this layer for some time showed evidence of general stellar collapse. The epidermis, though still intact, revealed considerable reduction of the cell contents. In needles like this, the activities of the tunnelling oribatid mites and other endophagous meiofauna had become possible. Needles were sectioned in which these animals had eaten out large cavities, usually in the mesophyll but occasionally encroaching on the vascular tissues, leaving behind their characteristic brown faecal pellets (Fig. 25).

Layer $F_2$ needles. Such needles of the $F_2$ layer as were sectioned seemed to have escaped extensive colonization by either *Fusicoccum* or *Desmazierella*, and showed a greater degree of collapse in the vascular tissues than in the mesophyll, in one case the stele having disappeared altogether (Fig. 26). These observations appeared to support the suggestion of HANDLEY (1954) that mor-forming litter showed more rapid decomposition of lignified than non-lignified tissues.

The dark surface reticula and conidiophores so prominent a feature of the $F_1$ layer remained only in traces, and were largely replaced by accumulations of dark material made up of faeces deposited by various types of ectophage, arthropod exoskeletal remains and other organic debris (Fig. 26). In addition the sections showed dark hyphae of the sterile form previously noted, both crossing the surface, and penetrating the interior of the needles.

The absence from many $F_2$ needles of extensive internal colonization by either of the main fungi of the succession suggested an interesting possibility. Infection of most needles by *Fusicoccum* and *Desmazierella* may have brought about considerable tissue softening and degradation. This softening would allow the entry of endophages and produce, as a result, a comparatively rapid and complete physical comminution of the needles. In the case of needles which had escaped completely or in part the action of the chief litter attacking fungi, the period elapsing before the tissues could be invaded and reduced by the fauna would, perhaps, be much longer. This might give rise to an accumulation of such needles and fragments of needles at lower levels of the organic horizon. It seems possible that the $F_2$ layer is, in part, such an accumulation.

## CLIMATIC EFFECTS ON THE SUCCESSION

### 1. *Temperature.*

Some soil fungi can show growth at —2° C with an optimum around 15° C, while a few grow quite satisfactorily at 37° C. During the period of this investigation, mean ground minima fell to freezing point only in February and December, (Table 5) while March was exceptionally warm. Mean ground maxima in unshaded situations in Britain may exceed air maxima by as much as 34° C on occasions, but OVINGTON and MADGWICK (1955) showed that only about 7 % of incident light penetrated a coniferous canopy, and WILKINS and HARRIS (1946) found such a canopy to reduce temperature variations to one-half of those found in the open. The extremes occurring in Delamere Forest during 1957 were a ground minimum of —9° C during February, and

Table 5

Meteorological Data

| Month | Precipitation | | Temperature °C | | | |
|---|---|---|---|---|---|---|
| | Delamere | Delamere | Delamere | | Chester | |
| | Total precipitation (mm.) | Consecutive days without rain | Air mean min. | Air mean max. | Ground mean min. | Earth mean |
| Jan. 1957 | 37.6 | 8 | 1 | 8.3 | 0.6 | 5 |
| Feb. | 63.2 | 4 | 0 | 8.3 | —1.7 | 4 |
| Mar. | 68.1 | 7 | 4.4 | 12.8 | 3.3 | 8.3 |
| Apr. | 13.7 | 10.9 | 2.2 | 15.6 | 1 | 10 |
| May | 33.0 | 10 | 4 | 18.6 | 4 | 13.3 |
| Jun. | 40.6 | 10 | 6.7 | 26 | 7.2 | 19 |
| Jul. | 118.9 | | 11 | 22 | 12.2 | 17.8 |
| Aug. | 87.9 | 4 | 10 | 21 | 10 | 17.2 |
| Sep. | 194.3 | | 7.2 | 17.2 | 7.2 | 14 |
| Oct. | 78.0 | 12 | 5.6 | 14.4 | 5.6 | 11.7 |
| Nov. | 46.0 | 5,9,10 | 2.2 | 9 | 2.2 | 7.2 |
| Dec. | 64.8 | 5,5 | 0 | 6.8 | —1 | 4.4 |
| Jan. 1958 | 77.2 | | | | | |
| Feb. | 110.7 | | | | | |
| Mar. | 51.3 | 11 | | | | |

Total 1085.3 mm.

Total reaching ground 750 mm. approx. assuming Wilkins & Harris' data to apply here.

The meteorological data were made available by the Forestry Commission of Great Britain, and by Dr. J. P. Savidge.

a ground maximum of 38° C in June. The latter figure is of particular significance, because in the absence of a canopy it might have been expected to exceed 60° C. The canopy appears to have been the only factor preventing a partial sterilization, at least of the L layer needles.

As distance below the surface increases, the temperature range and fluctuation recorded at ground level are reduced in magnitude. Earth mean temperatures recorded at a station near Delamere show that fungal growth in the subsurface layers of the organic horizon is unlikely to be completely inhibited by temperature at any time of year.

### 2. *Precipitation*

The moisture content of the litter is determined by rainfall and by the various factors which modify the subsequent evaporation process. Precipitation data for the experimental period are given in Table 5. However, not all the moisture represented by these figures reached the ground. Wilkins and Harris (1946) found that the canopy of a pine forest intercepted some 25 % of the total rainfall. Metz (1958) showed that after rain, litter of Loblolly pine continued to dry out for 11 days under average conditions before attaining equilibrium at 20 % moisture content. Webster (1956) found that mycelial growth within dead culms of *Dactylis glomeraia* was apparently inhibited if the substrate contained less than 15 % moisture. A moisture content for L layer needles lower than this figure was recorded only once, and it may be assumed that moisture rarely became limiting for fungal colonization of the internal tissues of the pine needles during the present study. Only on the exterior of the surface layer of needles, which were in direct contact with the atmosphere, did desiccation apparently cause inhibition of fungal growth.

## *The Meiofauna*

The meiofauna comprise a group of animals intermediate in size between the micro- and macro-fauna. Murphy (1955) proposed that the lower and upper size limits for this group be 100 μ and 1 cm. The activities of these animals may both precede and follow those of the fungi. Haddow (1941) found that damage on living, needles of red pine, originally caused by a Cecidomyiid gall midge, was colonized and extended by *Pullularia pullulans*. More recently Batko, Murray and Peace (1958) reported that the tunnelling activities of the pine gall midge, *Cecidomyia baeri*, in needles of Scots pine, had introduced the pathogenic *Sclerophoma pithyophila*. However, the fauna usually assume greater importance after the needles have fallen and been extensively colonized by the fungi. During the present study it became clear that while the $F_1$ layer was dominated by the fungi, the $F_2$ layer was the province of the meiofauna. It is well known that one of the main faunistic difference between mull and mor is the presence of large earthworms in the former and their absence from the latter. No earthworms were ever seen in the Delamere soil, but the fauna was nevertheless rich in species, and numerically very strong. Other workers have reported very large meiofaunal populations from mor-type organic horizons under conifers. FORSSLUND (1945) found over $10^6$ individuals per square metre to a depth of only 10 cm. or almost 11 animals to the cubic centimetre.

NIELSEN (1955) recorded maximum numbers of Enchytraeidae from coniferous plantations, with peak densities of up to $2 \times 10^5$ individuals per square metre. NIELSEN (1949) found $2.4$—$2.7 \times 10^6$ nematodes per square metre in the surface 5 cm. of a Danish mor. When it is appreciated that many of these animals rely for food either on fungal mycelia and spores, or on plant tissues softened by fungal action, their importance to the present study is clear. No quantitative studies were made, but animals seen during routine direct observations were collected, preserved, and submitted to specialist systematists for identification. A list of the most frequently collected animals is given in Table 6. The Oribatei or beetle mites were most frequently seen. OWEN-EVANS (1955) found that the Cryptostigmata, to which the Oribatei belong, formed a large proportion of the Acarina obtained from litter and subsoil of a coniferous plantation, and further that the Acarina comprised the majority of the Arthropod meiofauna. Both as ectophages and endophages, the mites have been seen to play an important part in the physical reduction of the pine litter. These animals were also among the most heavily sclerotised fauna, and were able to survive the dry conditions often encountered in the surface litter. RIHA (1944) found that *Pelops* and *Carabodes* were very resistant to desiccation, while *Adoristes*, *Ceratoppia* and *Chamobates* also possessed considerable drought resistance. Other less heavily sclerotised groups are restricted to the lower more moist layers of the horizon. Among these were the Collembola, dipterous larvae and enchytraeid worms collected during the study. Greatest numbers of animals were collected in the F layers. This agreed with the findings of DRIFT (1951) who studied the vertical distribution of animals in a mor-type organic horizon.

Both OWEN-EVANS (1955) and NIELSEN (1955), who followed the seasonal fluctuations in Arthropod and Enchytraeid meiofauna respectively, observed a summer depression in numbers, caused by such overriding factors as high temperatures and desiccation. This depres-

Table 6

List of the commoner Meiofauna found in the organic horizon at Delamere Forest

| | |
|---|---|
| Phylum: Annelida | |
| Class: Chaetopoda | |
| Order: Oligochaeta | |
| Family: Enchytraeidae | |
| | Fredericia spp. |
| Phylum: Arthropoda | |
| Class: Arachnida | |
| Order: Acarina | |
| Family: Oribatidae | |
| | Adoristes ovatus |
| | Carabodes minusculus |
| | Ceratoppia bipilis |
| | Chamobates incisus |
| | Odontocepheus elongatus |
| | Oppia unicarinata |
| | Pelops tardus |
| | Platynothrus peltifer |
| | Thyriosoma lanceolata |
| | Immature Oribatei. |
| Subphylum: Insecta | |
| Class: Apterygota | |
| Order: Collembola | |
| Family: Entomobryidae | |
| | Orchesella cincta |
| | Lepidocyrtus sp. |
| Class: Pterygota | |
| Subclass: Exopterygota | |
| Order: Hemiptera | |
| Family: Aphididae | |
| | Cinaria pinea |
| Subclass: Endopterygota | |
| Order: Diptera | |
| Family: Cecidomyiidae. | |
| | Cecidomyia baeri. |

sion was followed by an autumnal peak. It is believed that this autumnal peak was directly connected with the great reduction in numbers of conidiophores of various fungi in the F layer which was observed in Delamere at this time of year. The importance of the meiofauna was only fully realised, however, when the H layer was examined. This layer at Delamere consisted largely of their partially humified faeces. The first major phase of the decomposition was over. The subsequent fate of the material in the H layer must be the subject of another investigation.

## DISCUSSION

The several complementary techniques used during this study were developed in order to discover the nature of the micro-organisms which bring about the reduction of pine leaf litter to humus. In an earlier publication (KENDRICK, 1959) a method was described for determining the duration of the different stages of the decay. It is now possible to attempt a synthesis of the results obtained from the various approaches, and to suggest an overall scheme for the successional process of decomposition undergone by needles of *Pinus sylvestris* at Delamere Forest.

Although over seventy species of fungi were isolated or observed during this study, less than one fifth of these were encountered with sufficient frequency for their role in the breakdown to be accounted important. The occurrence of these fungi in space and time forms the subject of the following summary, and is diagrammatically represented in figure 27.

In spring about 40 % of living *P. sylvestris* needles on the tree become infected by *Lophodermium pinastri*, a parasitic ascomycete which produces no obvious symptoms at this stage. Approximately 50 % of living needles also bear a *Coniosporium* species whose presence was only revealed by cultural work. This seems to colonize only restricted areas of the needle surface, and its relationship to the living needle tissues is obscure. *Pullularia pullulans*, a common dematiaceous primary saprophyte, is present on the exterior of about 10 % of living needles in spring, but infects about 30 % in the months June to August. The sphaeropsidaceous *Fusicoccum bacillare* is found for the first time in May occurring in about 20 % of living needles. The needles attacked by these last two fungi are presumably senescent, and generally die and become brown while still attached to the tree. At this stage 80 % of these needles are infected with *Pullularia pullulans*, and 90 % with *Fusicoccum bacillare* while *Coniosporium* no longer appears in culture.

These needles fall to the ground in late August or early September, and become part of the L layer of litter, in which they spend on the average approximately six months. During this time the pycnidia of *Fusicoccum bacillare* and the hysterothecia and diaphragms of *Lophodermium pinastri* are produced, providing the inoculum whereby further living needles on the tree become infected. Meanwhile many of of the needles are invaded by *Desmazierella acicola*, a discomycete which was recorded during this study only in its *Verticicladium* conidial state. This species does not occur on living needles, but six to eight months after they have fallen it may be isolated from 70 % to 100 % of these same needles. Incidence of *Pullularia pullulans* and *Fusicoccum bacillare* remains high for three to four months, then begins to decline sharply in face of changed environmental conditions and, perhaps, increased competition from other fungi.

The next stage of the succession, represented spatially by the $F_1$ layer, is of longer duration, requiring on an average about two years for completion. During this phase the colonization of needles proceeds along two distinct lines. The interior of the needle is extensively attacked by *Desmazierella acicola*, which lays down zones of black pigment within the tissues at the limits of its growth, and produces numerous massive dematiaceous conidiophores through the stomates. Meanwhile the exterior of the needle, now surrounded by a much more humid atmosphere than that found in the surface litter, is colonized by two hyphomycetes, *Sympodiella acicola* and *Helicoma monospora* (Kendrick 1958a, 1958b) of which traces may often be found while needles are still in the L layer. These fungi produce appressed networks of fine dematiaceous hyphae, and later enormous numbers of small darkly pigmented conidiophores are formed. External and internal colonisers combine to bring about the darkening in colour which is such a characteristic feature of the $F_1$ layer. The conidiophores of *Desmazierella acicola* are first produced in summer, between 10 and 13 months after the needles have fallen, and subsequently, during the second year that needles spend in the $F_1$ layer, another and larger crop of conidiophores is formed. Conidiophores of *Sympodiella acicola* and *Helicoma monospora* are developed mainly in spring from 7 to 10 months after the needles have fallen, and again in the spring of the following year.

Because pine needles are very long in proportion to their thickness, the presence of one fungus in a needle does not preclude the occurrence in the same needle of other species. Most of the fungi encountered during this work do not show strong antagonisms or synergisms, and the timing of the different stages of the succession seems to be controlled mainly by environmental and nutritional factors. *Lophodermium pinastri*, however, by its production of pigmented diaphragms across the needle, often creates an impenetrable barrier to the growth of other internal colonizers, especially *Desmazierella acicola*, along the needle. This results in adjoining sections of the same needle being decomposed at different rates and by different combinations of microorganisms.

After approximately two years in the $F_1$ layer, the character of the needles changes once more. They become tightly compressed and generally break up into fragments. Those fragments which have been attacked internally by *Lophodermium*, *Fusicoccum* and *Desmazierella* are gradually penetrated by members of the soil fauna which are the dominant organisms of this phase. Ectophages—mites, collembola, enchytraeid worms and others—remove the innumerable conidiophores of *Helicoma* and *Sympodiella*, attaining maximum activity in spring and autumn. Meanwhile endophages, frequently mite instars, eat their way through the interior of the needles, eventually bringing about complete comminution. Those fragments which have escaped extensive internal attack by fungi accumulate in the $F_2$ layer. *Trichoderma viride* and one or more species of *Penicillium* occur on most of these, but it seems probable that these fungi are usually present only as a high spore potential.

The needle fragments are slowly attacked by basidiomycetes and a sterile dematiaceous form over a period of about 7 years. Eventually the meiofauna complete their physical reduction also, and the remains enter the H or humus layer.

In this layer the pine needles are no longer recognizable, and so the time required for the ultimate breakdown of the humus cannot at present be accurately assessed. What does seem certain, from the very acid conditions, the accumulation of amorphous humus, and the apparent inactivation or death of most fungi, is that biological activity has now fallen to a very low level.

It appears that pine needles exert a very strong selective effect on would-be colonizers, and many of the fungi participating in the succession are uncommon or host-specific. Accordingly, little is known of their physiology, but it does seem fairly clear that the 'sugar fungi' (Burges, 1939) with the exception of *Pullularia pullulans*, are conspicuously absent from the early stages of the breakdown.

Although the present study has gone some way towards elucidating the fungal succession involved in the decay of Scots pine needles at Delamere Forest, it is not claimed that the results obtained here can be applied directly to other locations even under the same tree cover.

Climatic or site differences may lead to variation in the form of the humus; similarly, where different species of plants form the litter, marked changes may be expected in the fungi involved in the succession as preliminary observations on the litter of *Pinus nigra* var *corsicana* have already shown. Nevertheless the general pattern of decay on mor sites such as described in this work, and the complementary rôles played by the fungi and the animals during the succession, are now well established. The fungi perform an initial softening and predigestion of the needle tissues, and the animals complete the process of chemical and physical reduction to humus.

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## EXPLANATION OF PLATES 65 (1)–78 (14)

### Plate 65 (1)

Fig. 1. Drawing of a vertical section through the litter, (obtained by embedding undisturbed litter in cold-setting resin and sectioning with a diamond-edged geological wheel) showing spatial relationships of needles in the different layers, and the progressive reduction in size of interstices with increasing depth.

### Plate 66 (2)

Figs. 2 & 3. Percentage occurrence in culture of the fungi most frequently isolated from washed and surface sterilized living needles round the year. Occurrence was recorded simply as presence on a needle, extent of colonization being disregarded.

### Plate 67 (3)

Figs. 4 & 5. Percentage occurrence in culture of the fungi most frequently isolated from washed and surface sterilized needles of the L layer round the year. In these two figures, o denotes 'old' needles, and n denotes needles added to the L layer by the seasonal needle fall. See Fig. 3 for legend.

### Plate 68 (4)

Figs. 6 & 7. Percentage occurrence in culture of the fungi most frequently isolated from washed and surface sterilized needles of the $F_1$ layer round the year. See Fig. 3 for legend.

### Plate 69 (5)

Figs. 8 & 9. Percentage occurrence in culture of the fungi most frequently isolated from washed and surface-sterilized needles of the $F_2$ layer round the year. See Fig. 3 for legend.

### Plate 70 (6)

Fig. 10. Needle samples of the types recognized during this study—A, $L_n$, $L_0$, $F_1$ and $F_2$—in the screw-top phials used in the washing technique. × ¾.

Fig. 11. Direct observation—Hysterothecia of *Lophodermium pinastri* on a needle of the L layer. × 18.

Fig. 12. Direct observation—First traces of the superficial hyphal networks of *Helicoma monospora* and *Sympodiella acicola* on an 'old' needle of the L layer. × 80.

### Plate 71 (7)

Fig. 13. Direct observation—Reticulum and conidiophores of *Helicoma monospora* on a needle of the $F_1$ layer. × 110.

Fig. 14. Direct observation—Hyphal reticulum of *Sympodiella acicola* on a needle of the $F_1$ layer. × 1140.

Plate 72 (8)

Fig. 15. Direct observation—Conidiophores of *Sympodiella acicola* growing on a needle of the $F_1$ layer. × 240.

Fig. 16. Direct observation—Massive conidiophores of *Desmazierella acicola* growing out from a needle of the $F_1$ layer. × 50.

Plate 73 (9)

Fig. 17. Direct observation—A 'diaphragm' of *Lophodermium pinastri* prevents further growth by *Desmazierella acicola* along a needle of the $F_1$ layer. × 30.

Fig. 18. Direct observation—a squash preparation of the H layer, showing the presence of undecomposed conidiophores of (A) *Desmazierella acicola*, (B) *Helicoma monospora*, and (C) *Sympodiella acicola*. × 150.

Plate 74 (10)

Fig. 19. Transverse section of a needle from the L layer, passing through two pycnidia of *Fusicoccum bacillare*. × 80.

Fig. 20. Transverse section of a needle from the L layer, passing through two hysterothecia of *Lophodermium pinastri*. × 80.

Plate 75 (4)

Fig. 21. Transverse section of a needle from the L layer, passing partly through a 'diaphragm' of *Lophodermium pinastri*. × 120.

Fig. 22. Section through the surface layers of a needle from the L layer, showing two stromata in the substomatal cavities from which conidiophores of *Desmazierella acicola* later arise. × 400.

Plate 76 (12)

Fig. 23. Transverse section of a needle from the $F_1$ layer through a zone of *Desmazierella acicola* colonization, showing the darkly pigmented boundary and three conidiophores. × 90.

Fig. 24. Section through the surface layers of a needle from the $F_1$ layer, showing conidiophores and dematiaceous hyphal layer of *Helicoma monospora*. × 580.

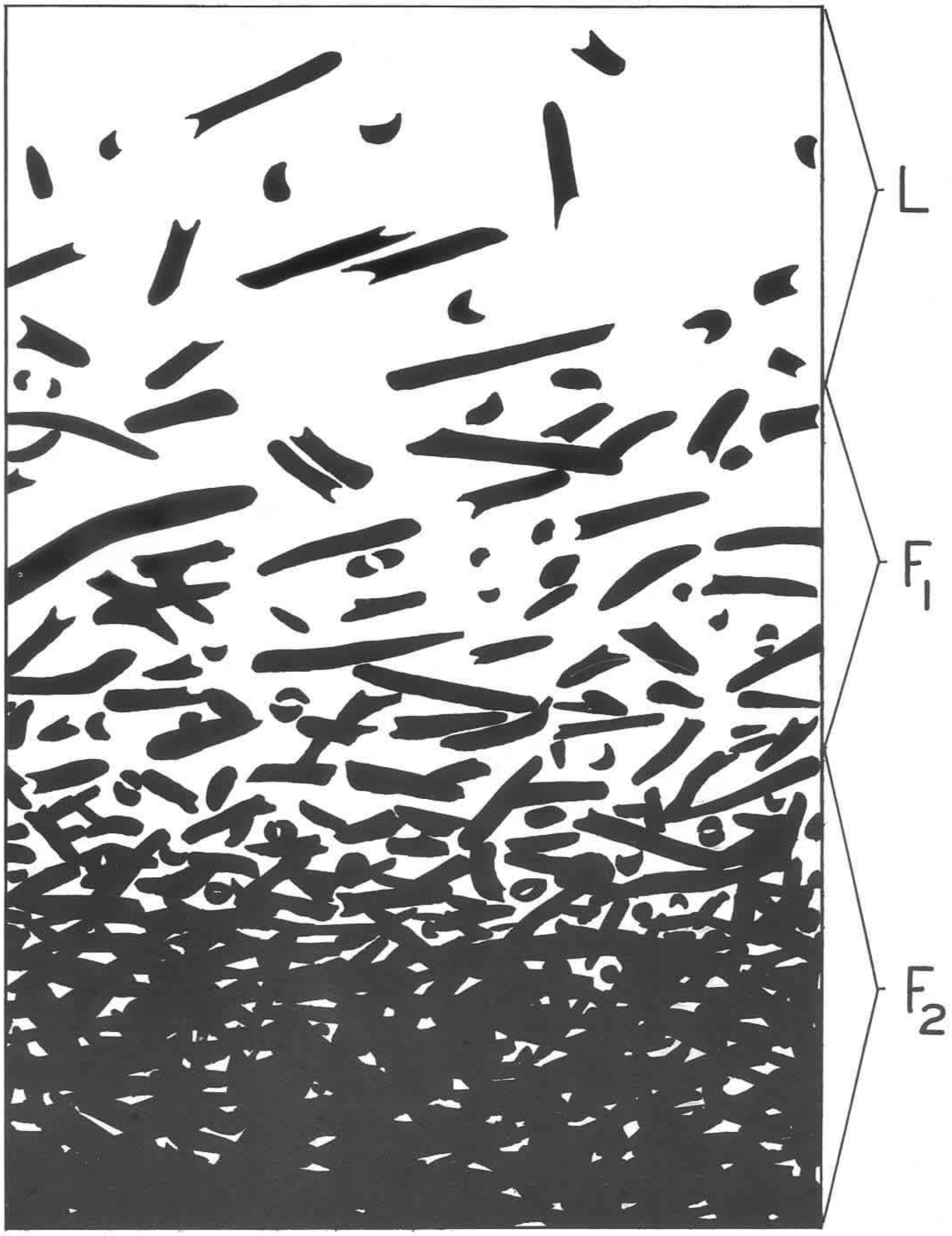
Plate 77 (13)

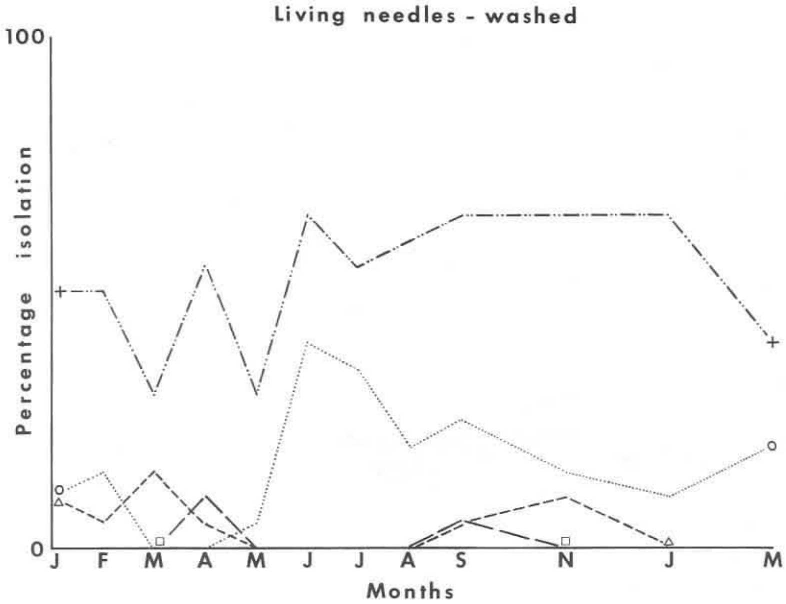
Fig. 25. Transverse section of a needle from the $F_1$ layer showing a cavity eaten out by an endophagous mite. The ovoid bodies are the animal's faecal pellets. × 140.

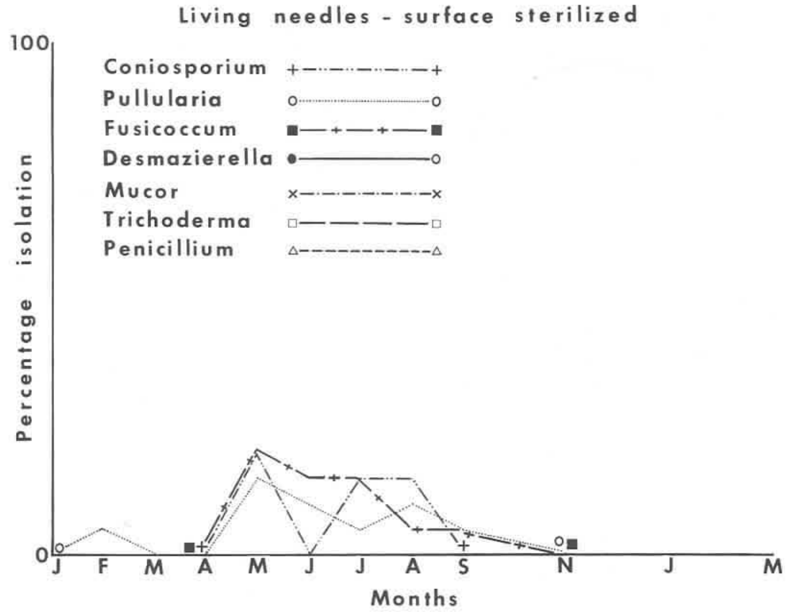
Fig. 26. Transverse section of a needle from the $F_2$ layer showing complete collapse of the stele, and surface accumulations of ectophagic debris. × 75.

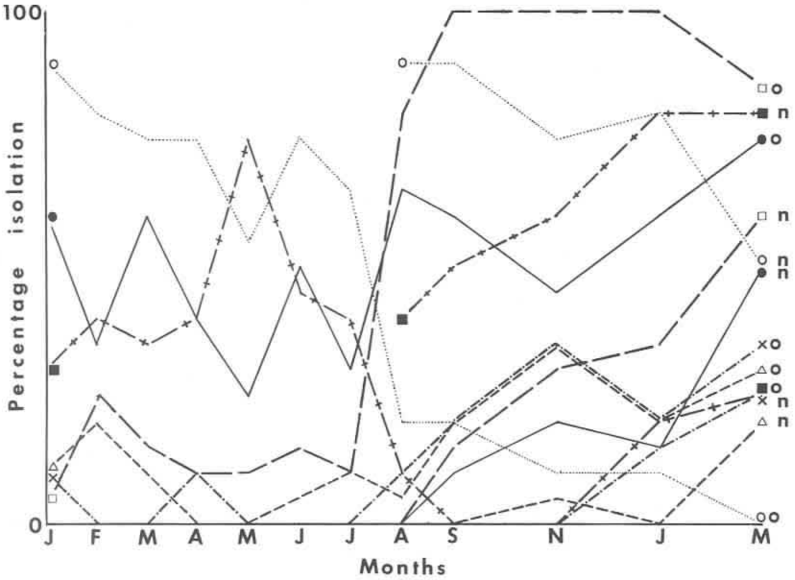
Plate 78 (14)

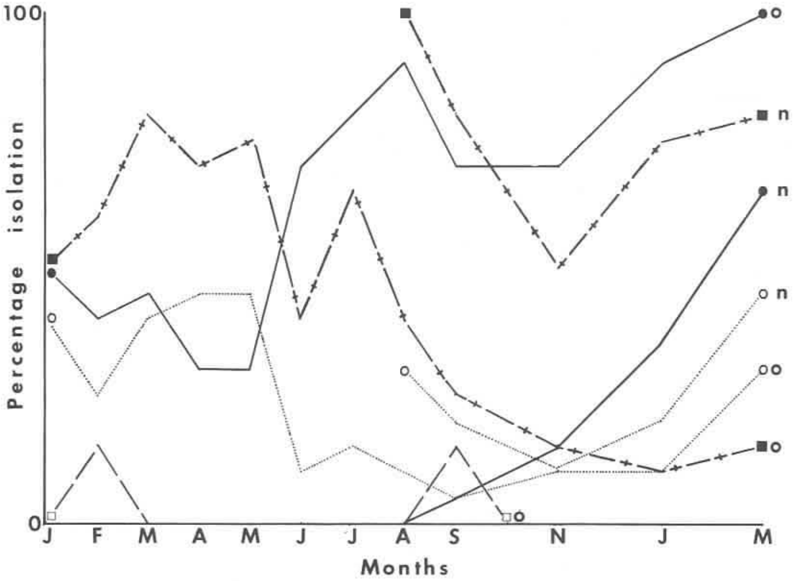
Fig. 27. Generalized schematic representation of the fungal succession, showing the temporal and spatial relationships of the fungi mainly concerned in the breakdown process.

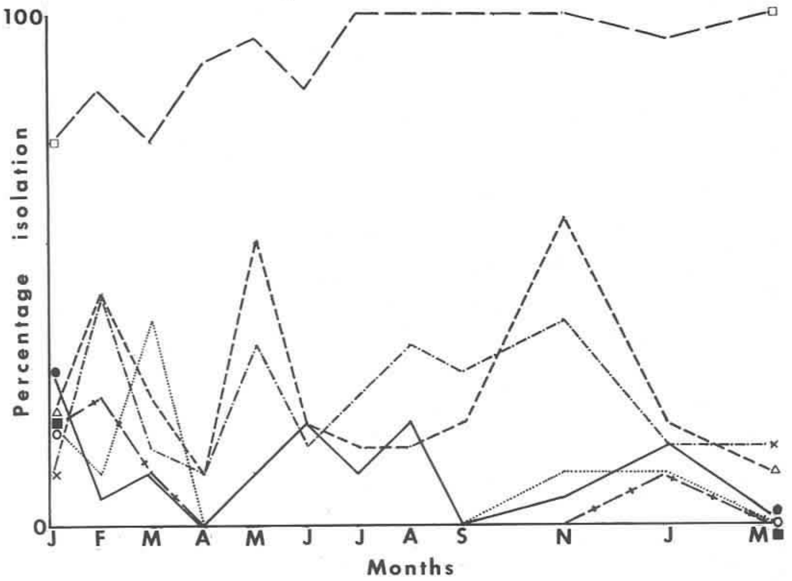

L
$F_1$
$F_2$


Living needles - washed

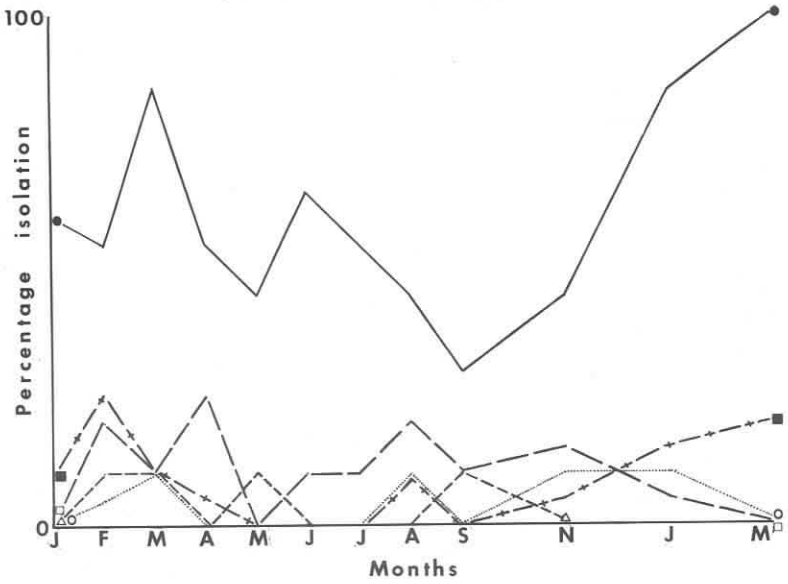

Living needles - surface sterilized


'L' needles - washed


'L' needles - surface sterilized

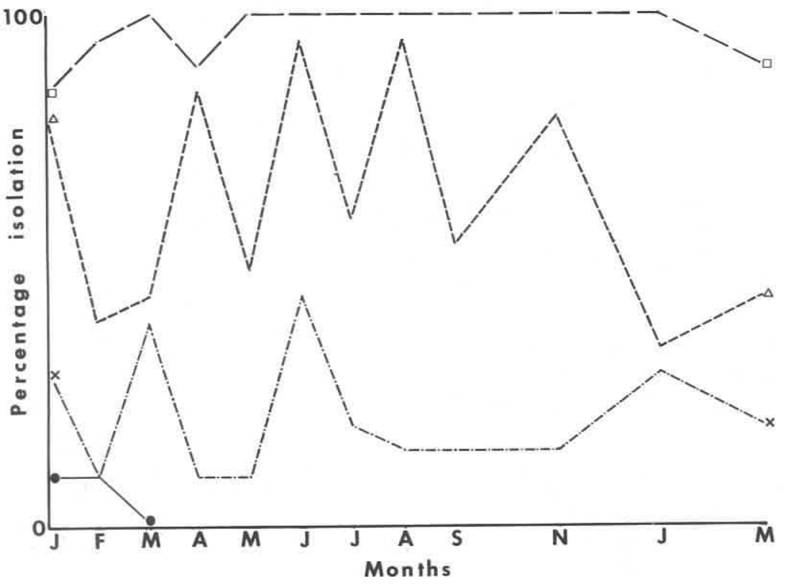


'F₁' needles - washed

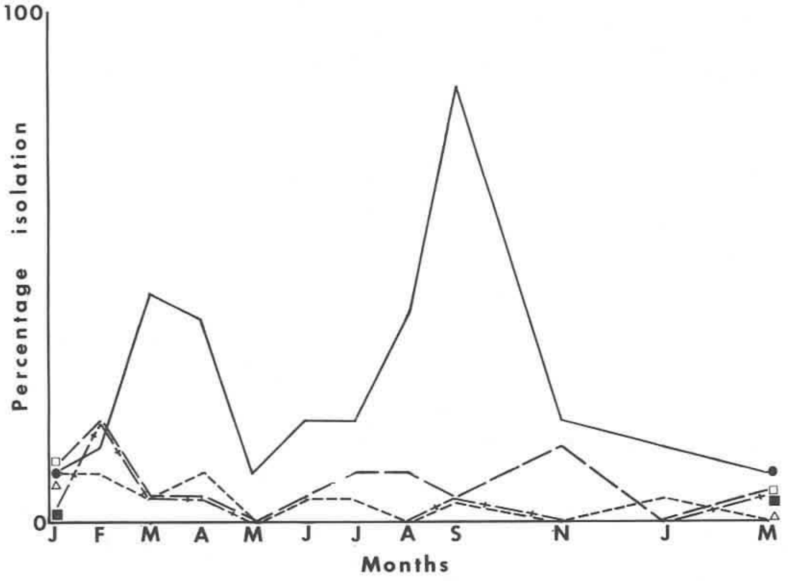


'F₁' needles - surface sterilized

'F$_2$' needles - washed



'F$_2$' needles - surface sterilized



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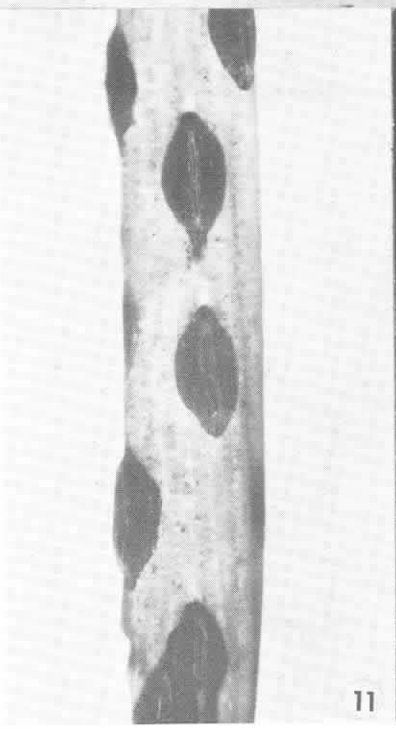

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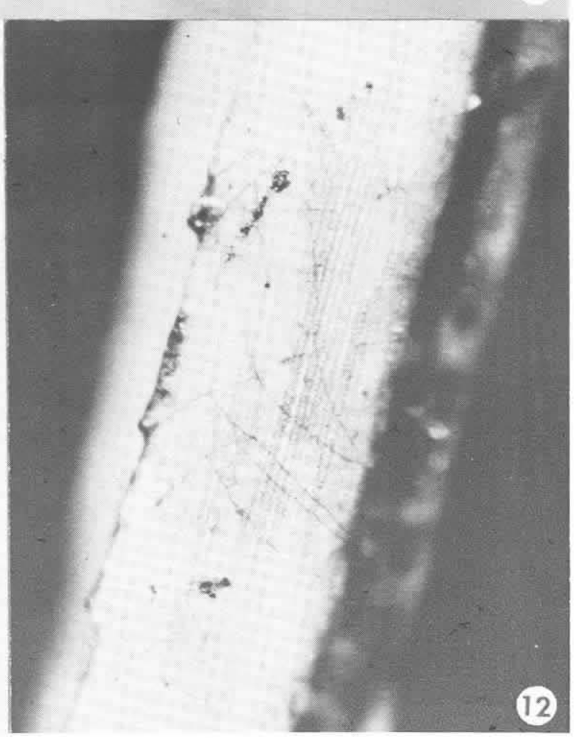

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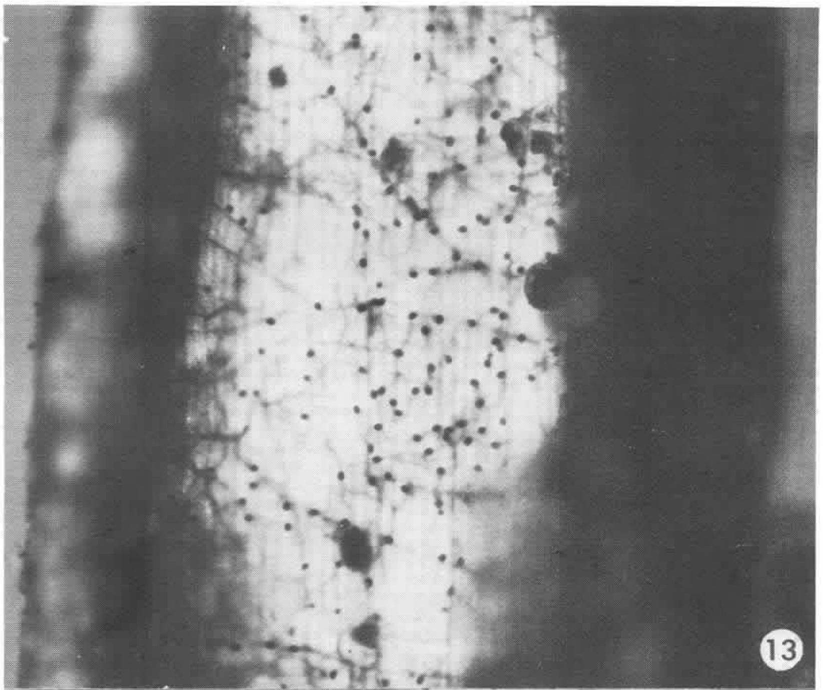

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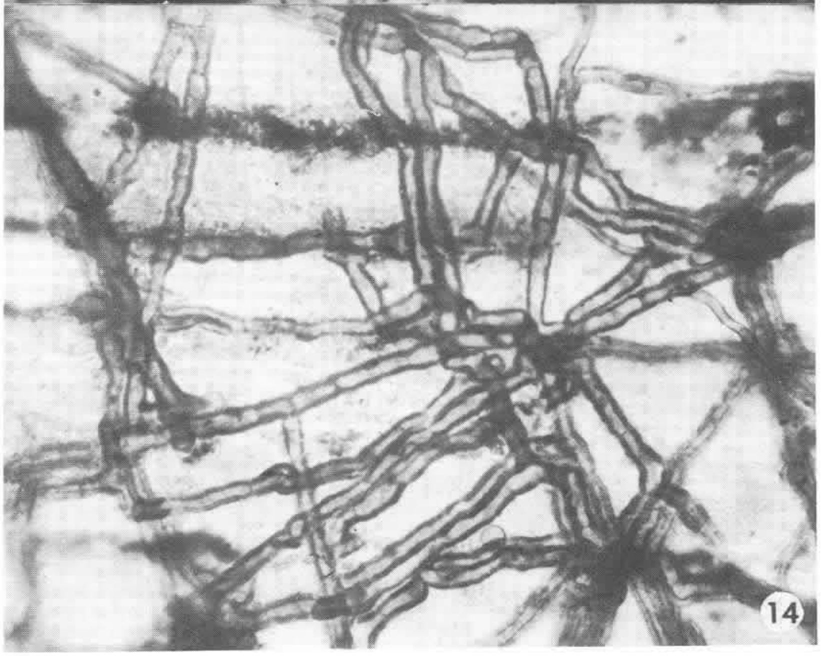

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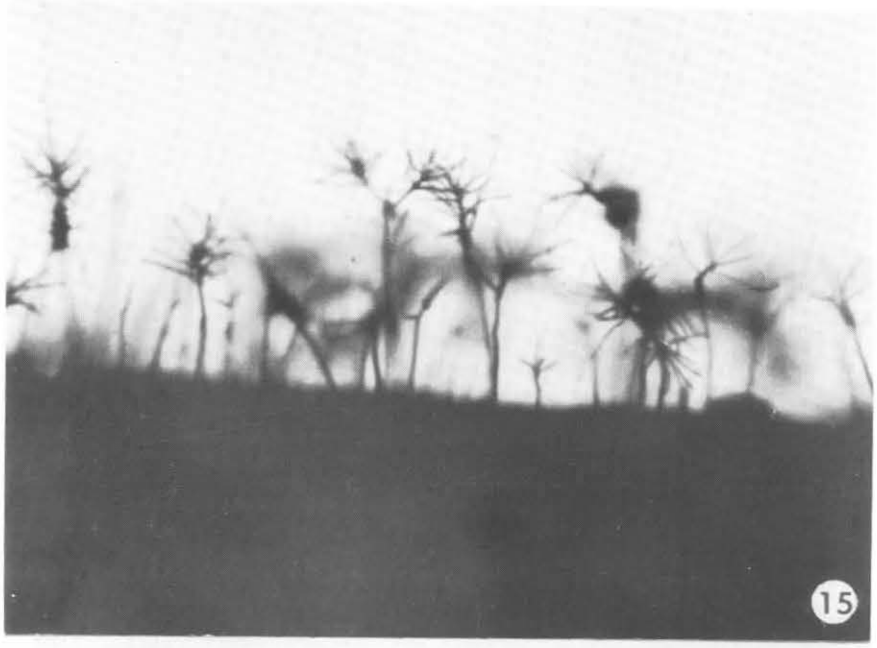
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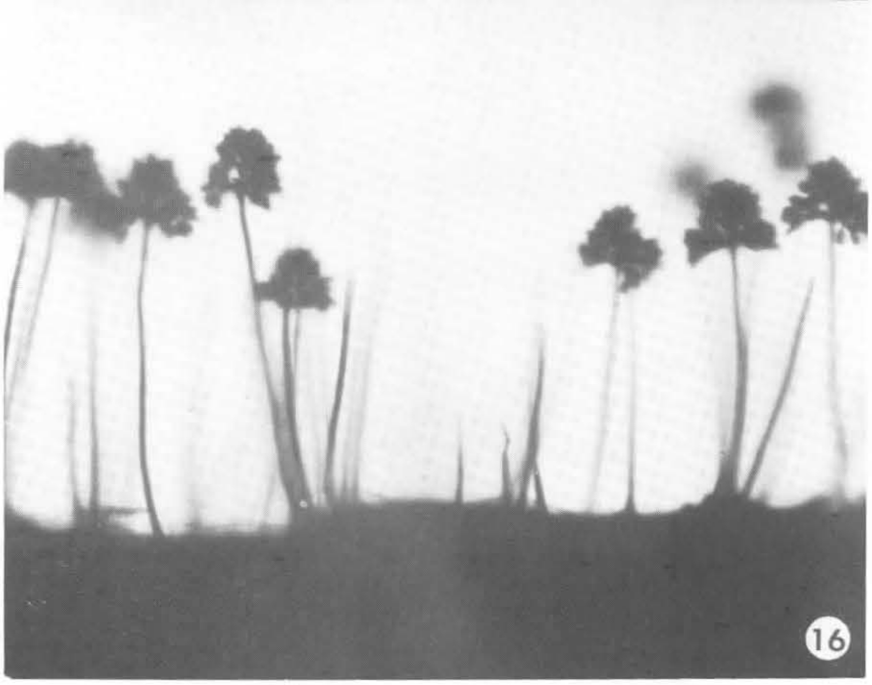
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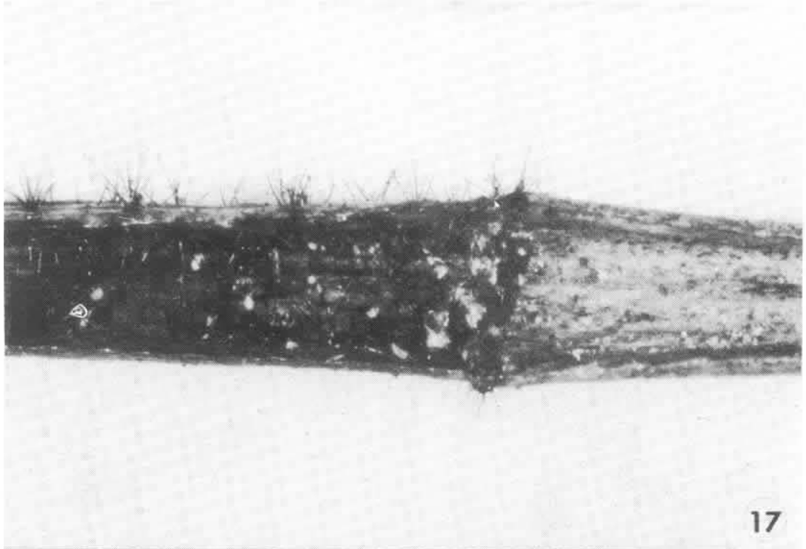

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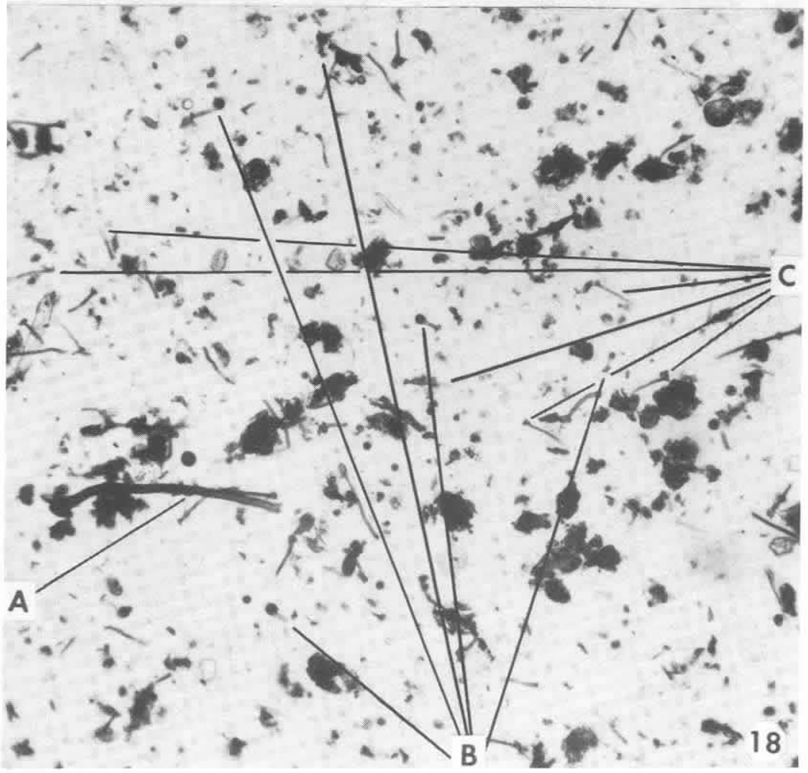

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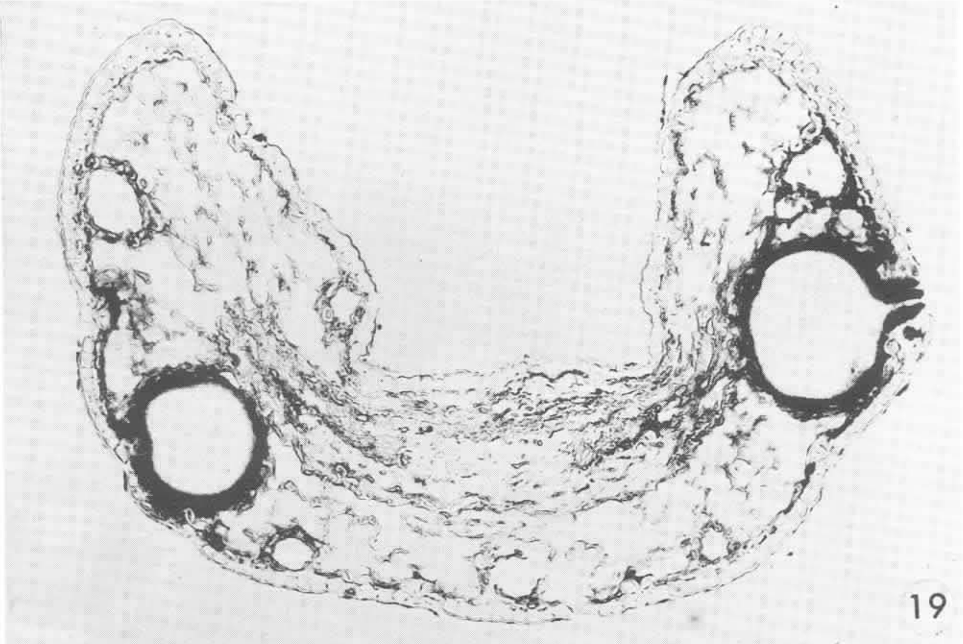

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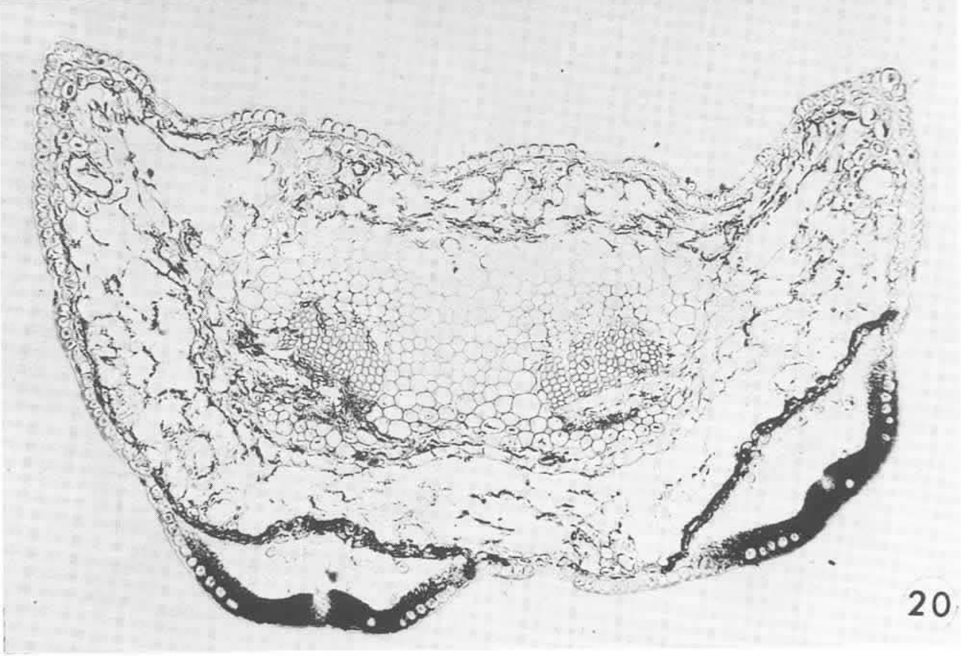

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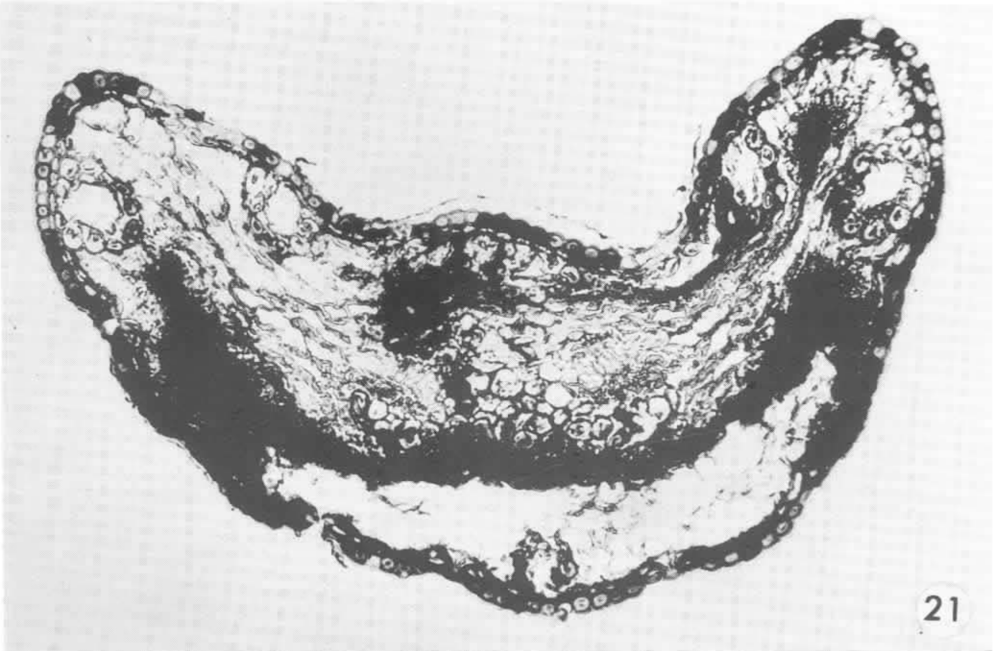

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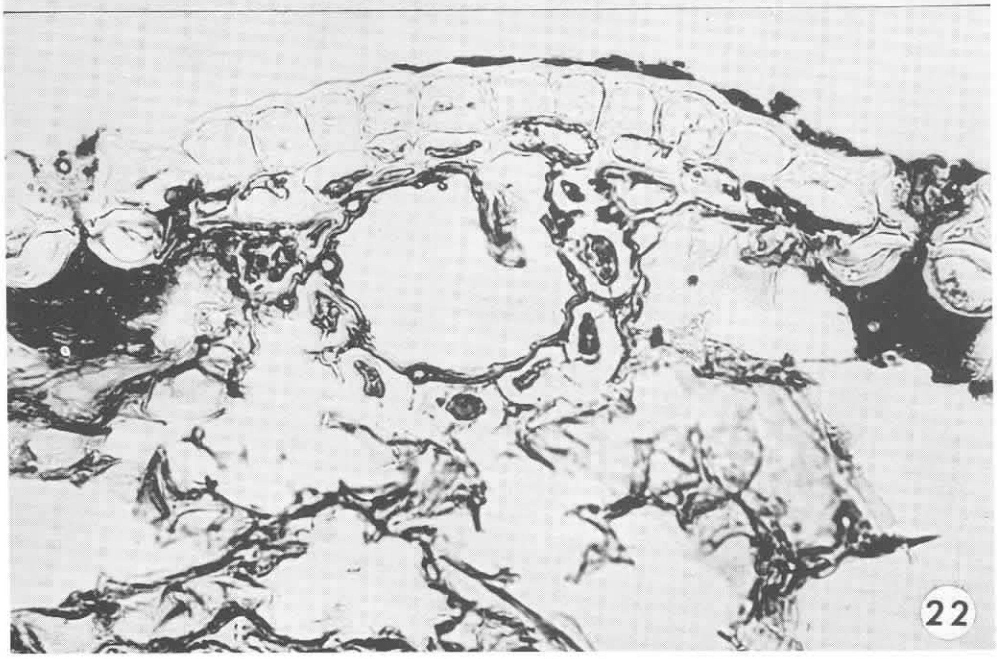

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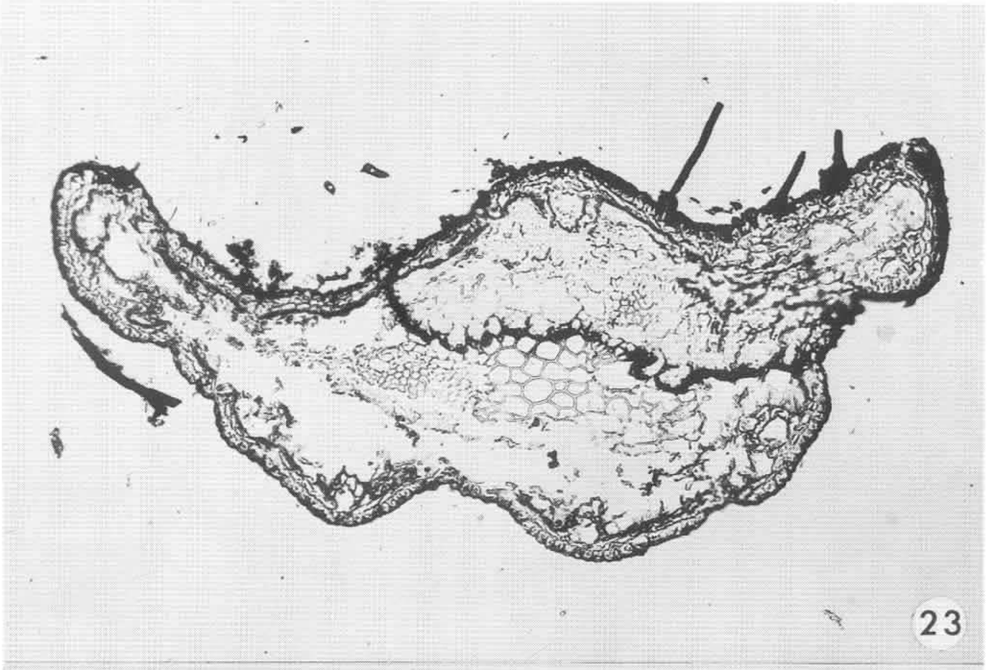

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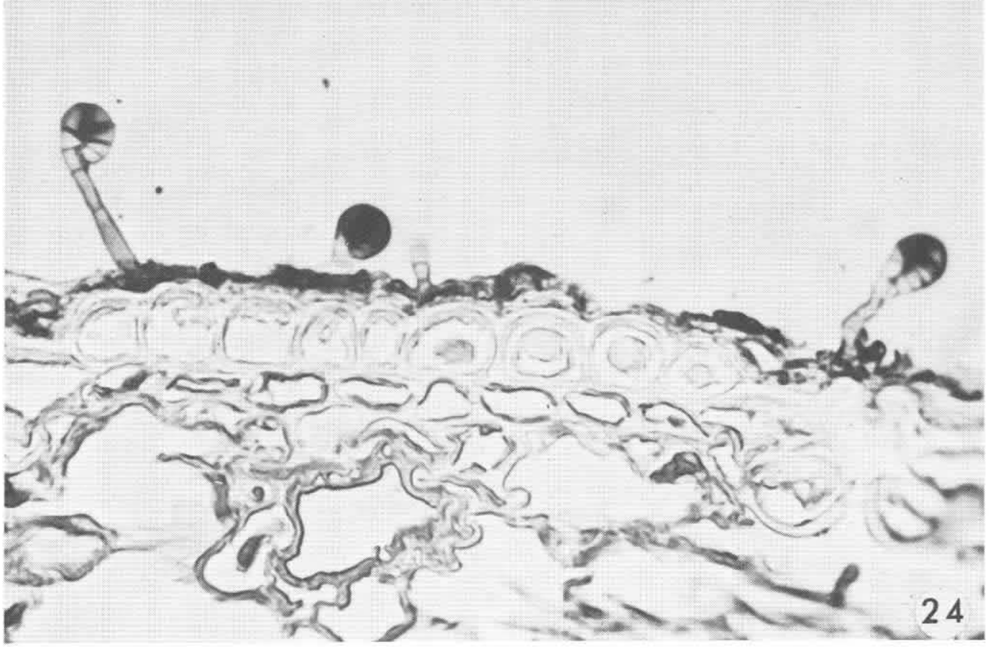

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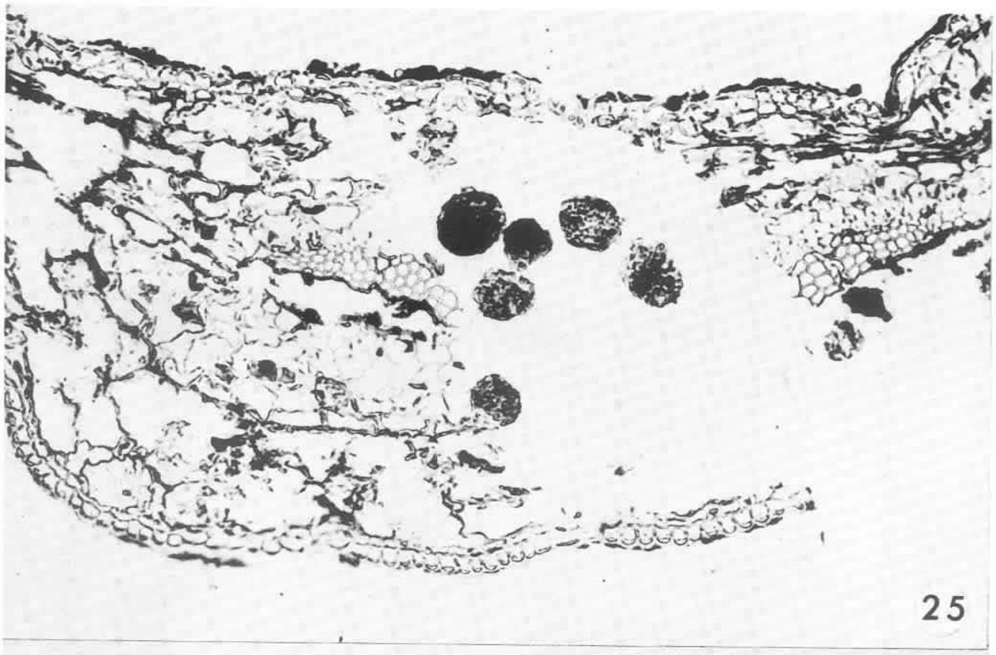

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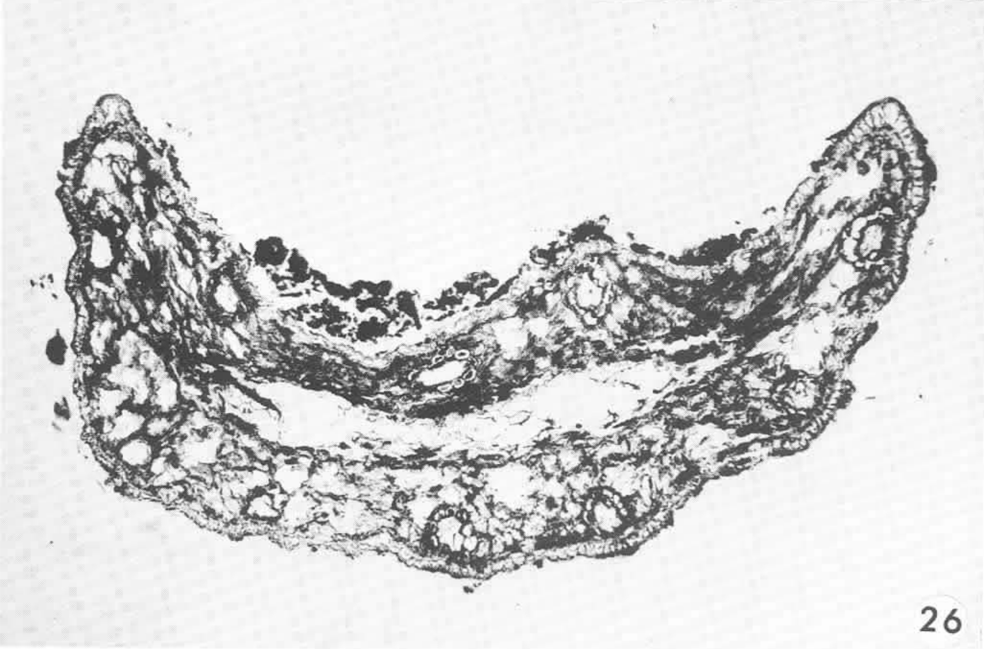

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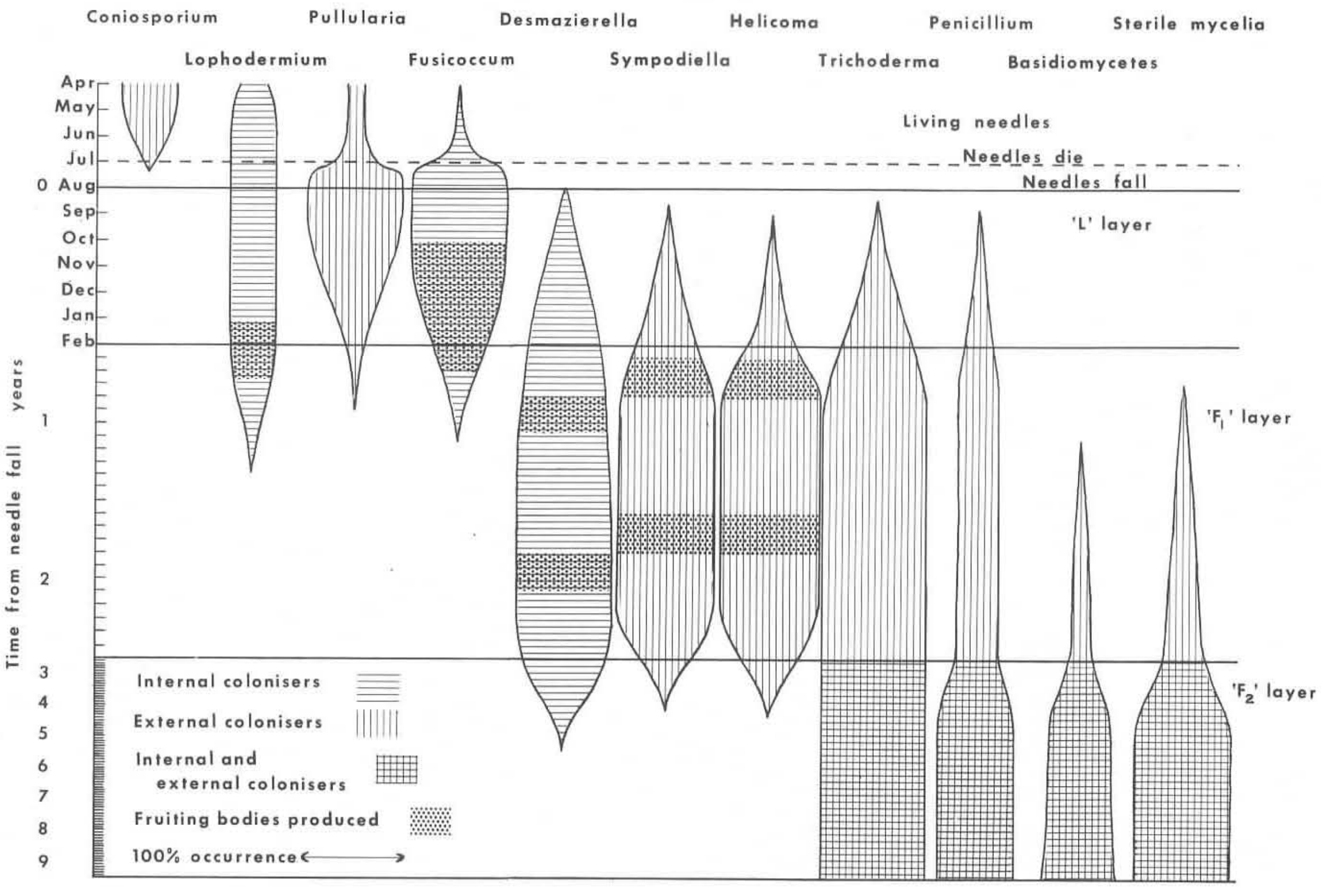
Coniosporium
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Pullularia
Fusicoccum
Desmazierella
Sympodiella
Helicoma
Trichoderma
Penicillium
Basidiomycetes
Sterile mycelia
Apr
May
Jun
Jul
0 Aug
Sep
Oct
Nov
Dec
Jan
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1
2
3
4
5
6
7
8
9
Time from needle fall years
Living needles
Needles die
Needles fall
'L' layer
'F1' layer
'F2' layer
Internal colonisers
External colonisers
Internal and external colonisers
Fruiting bodies produced
100% occurrence